Thesis for D. Sc.

PATHOLOGICAL STUDIES ON SPLENOMEGALY.

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VENOUS SPLENOMEGALY.

A Study in Experimental Portal Congestion.

INTRODUCTION.

The effect of venous congestion in the production of chronic splenomegaly in man has been variously estimated. Although experimental work on this problem has not met with any conclusive results, it has been argued that the extreme splenomegaly of splenic anaemia and Banti's disease is the result of primary obstructive lesions of the portal and splenic veins. (Dock & Warthin, 1904; Warthin, 1911; Klemperer, 1928, 1936). On the other hand, the fact that the spleen in cardiac stasis never shows such extreme enlargement unless there is a complicating factor such as subacute endocarditis, is against this view.

Of pioneer studies on the circulatory disturbances of the spleen, Malpighi's experiment of ligation of the vessels of the spleen in a young dog (Malpighi, quoted by Foster, 1901) was followed by an atrophy of the organ. Ligation of the portal vein has also been carried out, but mostly with a view to determine the effect on the liver. Thus Moos (1859) describes a shrinkage of the liver and an increase of connective tissue three months after ligature of the portal vein in/

in frogs and rabbits. Solowieff (1875) found that experimental blockage of the portal vein, if complete, was followed by death in 4 to 22 hours. Ligature of the splenic or superior mesenteric veins was however not fatal. A gradual blockage of the portal vein was followed by interstitial fibrosis of the liver. Cohnheim and Litten (1876) however could not confirm these results. They held that atrophy and destruction of the lobules of the liver could not be brought about by obstruction to the large branches of the portal vein, but only by obstruction of the interlobular veins. With regard to splenic changes, a congestive reaction following blockage of the splenic vein was first noted by Basler (1863), and the further studies of Sokoloff (1888) defined the congestive reaction. He found that in the early stages of congestion in dogs and rabbits, within about ten minutes after obstruction, there was a distension of the pulp veins and of the sinuses, but that as the congestion increased there was a percola-:tion of blood into the meshes of the pulp which he argued was due to the alteration of a "closed" to an "open" circulation. Wicklein (1891) found in experi-:ments in dogs that venous engorgement produced by constriction of the veins gradually disappeared within 21 days after the operation. The limitations of the congestive enlargement were determined by Warthin (1911) who/

who found that after ligature of the splenic veins in dogs and rabbits there was an immediate passive congestion with moderate enlargement of the spleen; this lasted for about six weeks and was followed by irregular atrophy of the spleen. No evidence of proliferative changes could be obtained. Warthin concluded that the condition of splenic hyperplasia in man due to obstruction by thrombosis of the splenic and portal veins could not be reproduced in the experimental animal. In experiments in rabbits by ligature of the main branch of the portal vein, Rous and Larrimore (1920) have not recorded any significant splenic changes. They found an extensive atrophy of the corresponding lobes of the liver and an associated hypertrophy of the lobes where the blood supply was still intact. Steenhuis (1911) had earlier recorded similar hepatic changes.

Of recent experiments directly related to the problem, are the studies of Jäeger (1931) in dogs. After partial ligature of the portal vein he claimed that the effect was quite different from that induced by ligature of the splenic vein since an intermittent congestion was induced by the portal tide which varied with the alimentary absorption. Histologically, Jäeger claimed that the capsulo-trabecular system showed predominant changes in congestion induced by obstruction/

obstruction of the splenic vein, while portal vein occlusion was followed by distension of the sinuses. He held that the morbid picture of the "fibro-adenie" of Banti could be induced merely by congestion. An analysis of his experimental work, brings out the important fact that in none of these cases was there any evidence of definite proliferative reactions or gross splenomegaly.

In order to determine whether proliferative reactions could be induced in the spleen by portal congestion it was necessary to induce varying stages of portal obstruction and study the effects on the spleen.

MATERIAL AND METHODS.

It was decided to use rats and rabbits as they had already been used for the production of cirrhosis. A larger number of experiments could be carried out, even though operative procedures are rendered more difficult by the smaller size of the vessels. Experimental congestion by ligature of the splenic vein has the obvious disadvantage that a collateral circulation from the gastric vessels would soon be established and render a chronic congestion difficult. Besides, a portal obstruction would offer for comparison conditions related to the later stages of cirrhosis and Banti's disease. It would be possible to allocate the effects of portal stasis as different from a toxic factor in the splenomegaly of cirrhosis.

The following methods of inducing obstruction were carried out: 1. Complete closure of the portal vein at the gastro-hepatic omentum by a silk ligature. 2. Partial obstruction of one third to two thirds of the diameter of the vein by a small elastic band or a loop of silk thread. 3. Partial obstruction of the vein by a kink produced by a loop attached to the under surface of the liver. 4. Obstruction of the left main branch by a ligature close to the porta hepatis. 5. Partial obstruction of the vein induced by/

by means of a pituitary clip clamped on one side of the vein so as to induce various degrees of constriction.

6. Obstruction by experimentally induced portal phlebitis and phlebosclerosis by the action of caustics on the portal vein.

Anatomical Considerations.

The portal vein of the rabbit is peculiar in that it is continued to the under surface of the liver as the main left branch while a smaller right branch is given off before reaching the liver. This supplies the right posterior and caudate lobes. The right anterior, the left anterior and posterior lobes together form the main bulk of the liver which is supplied by the main left branch. The common bile duct lies immediately on the ventral surface of the portal vein a little to the right with the hepatic artery deeper down and more to the right of the vein.

In the rat the vein is very thin and small, but otherwise the relations are more or less similar.

Operative Technique.

Under ether anaesthesia the abdomen was opened by a median incision extending from the xiphoid cartilage vertically down for an inch and a half. The anterior margin of the liver was kept up by means of a retractor, the/

the lower border of the stomach was then pulled out to bring out the gastro hepatic omentum. A curved director was then passed from behind forwards through the omental fold to include the vein without tearing through the wall. The hepatic artery and common bile duct were gently dissected off by a blunt needle and a loop of the ligature material was then passed through and dealt with in one of the ways mentioned above. For ligaturing the main left branch it was necessary to ligature high up close to the under surface of the liver beyond the smaller right branch. When a pituitary clip was used, the operation of partial clamping was easily carried out since the clip could be slipped on from the right side over the omental fold, if care was also taken to see that the hepatic artery and common bile duct were not included. The clamping was easily carried out by a special clip forceps designed by Mr. Norman Dott for his pituitary operations. It was found on subsequent operation that, if the clip was well clamped down, there was no danger of its slipping. The abdominal wound was then sutured in two layers.

Those animals that survived the operations were killed after periods varying from 2½ months to six months. The spleen was removed entire, with the splenic vessels and measured and weighed before fixa-:tion. In most of the cases, both the spleen and

pieces/

pieces of the liver were histologically examined. After fixation in Helly's fluid, paraffin slides were stained by (1) Mayer's haemalum and eosin; (2) Anderson's iron haematoxylin and Van Gieson's stain; (3) Heidenhain's azan stain, and (4) Wilder's modification of the Foot-Bielschowsky stain for reticulum.

RESULTS.

General Effects.

Some animals died in the earlier operations as a result of haemorrhage from puncture of the vein. This occurred when the director was passed from behind, as the vein is a very thin walled structure in these animals. Only one of the operated rabbits developed sepsis. This was however quite superficial and from infection of the suture material. In all the animals operated on portal congestion was well defined even six months after, as could be judged by the condition of the splenic veins at autopsy as well as by the condition of the trabecular veins and pulp veins in histological sections. As a rule, complete occlusion of the portal vein was invariably fatal in rats and rabbits, death taking place from three hours to four days. With partial occlusion the animals survived and remained apparently in good health. It was noticable in rabbits, that within a few days after the operation the abdominal veins became distended and very prominent. This effect gradually passed off, but the veins remained more prominent than normal.

The Effect of Complete Portal Obstruction.

The effect of complete ligation of the portal vein in/

in rats and rabbits was invariably fatal, in from 3 to 48 hours in rats and in 20 hours to 4 days in rabbits (see protocols I and II). At autopsy, it was found that death was due to mesenteric thrombosis, followed by rupture of the venous tributaries into the intestine and fatal haemorrhage. The post mortem appearances were remarkably constant. The mesenteric veins below the ligature were congested and engorged and the splenic vein was distended and swollen. The coils of the jejunum and the upper coils of the ileum were dark purple in colour and the smaller venous twigs of the mesentery and in the subserous coats of the intestine were all turgid. The lumen of the intestine was filled with clotted blood. The spleen was enlarged to about three times the normal size as shown in histograms I and II. It was dark purple in colour, the capsule was tense, almost like a bladder and on section, the cut surface oozed blood. The liver was generally pale and flabby but no other changes could be demonstrated.

Histologically the changes in the spleen were constant and varied only in degree.

In rats dying from 3 - 12 hours after the operation the condition of the spleen resembled that met with in venous infarction. The capsule appeared as an attenuated layer of fibrous tissue which had lost all wavy wrinkling and/

and looked like the distended wall of a bladder; the trabeculae were very thin and trabecular branching was almost indistinguishable except by the azan stain. The congestion was so marked that the cytoplasmic reticular syncytium was almost completely obscured and only the attenuated remnants of a mesh work could be made out by Van Gieson's and Heidenhain's azan stains. The protoplasm of the pulp syncytium had disappeared in many places and only a few drawn out threads remained. In places where haemorrhages had taken place, the syncytium had undergone necrosis, a change that is reminiscent of the venous infarctions met with in the cerebral cortex following thrombosis of the cortical veins. Owing to the distension of the pulp mesh with blood, only a few groups of cellular clusters could be made out. Some of these were the compressed malpighian follicles; others were outlying islands of lymphoid tissue which are normally present in the rat's spleen. The malpighian follicles had retained their basic structure, but the three well defined zones that are normally present in the rat's spleen were all compressed into one cluster of lymphoid cells while the lymphoid reticulum was hardly visible. On the whole the appearance of the spleen was that of a few/

Protocol of Experiments I.

Obstruction of the Portal Vein: Rats.

Animal No.	Body weight in gm.	Size of spleen in c.m.	Spleen weight in gm.	Estimated normal weight	Weight ratio (enlargement)	Nature of obstruction	Duration Remarks
P.L.I	220	4.2-1.1-.7	1.26	.59	2.1	complete ligature	6 hrs
P.L.III	150	3.7-8-.5	.64	.41	1.6	complete ligature	3 hrs
P.L.II	160	4-1.1-.5	1.24	.45	2.8	complete ligature	6 hrs
P.L.V	230	4-1-.7	1.36	.62	2.2	complete ligature	12 hrs
P.L.VI	180	4-1.1-.8	1.23	.49	2.5	complete ligature	12 hrs
P.L.VII	210	4.3-1.1-.7	1.60	.57	2.8	kinking	12 hrs venous infarction
P.L.VIII	180	3.8-1.3-.5	1.21	.49	2.5	kinking	48 hrs venous infarction
P.L.XI	230	4.4-1.3-.4	1.96	.62	3.1	partial ligature	12 hrs venous infarction
P.L.X	250	3.8-.9-.3	.82	.68	1.3	elastic (partial) ligature	2 months
P.L.IV	200	3.5-.9-.3	.79	.54	1.5	elastic (partial) ligature	4 months
P.L.IX	150	2.9-.5-.2	.35	.41	.9	ligation of right branch	10 days
P.P.I	200	2.8-.8-.3	.54	.54	1.0	portal phlebitis	4 months
P.P.II	250	3.5-.9-.35	.82	.68	1.2	portal phlebitis	4 months

Protocol of Experiments. II.

Obstruction of the Portal Vein: Rabbits.

Animal No.	Body weight in gm.	Size of spleen in c.m.	Spleen weight in gm.	Estimated normal weight	Weight ratio (enlarge-:ment	Nature of obstruc-:tion	Duration Remarks
P.L.IV	1180	3.8-.5-.3	.79	.59	1.31	complete ligature	20 hrs
P.L.VIII	960	6.5-1.2-.3	1.51	.48	3.1	complete ligature	24 hrs
P.L.X	920	4-1-.3	.98	.46	2.1	complete ligature	4 days
P.L.VI	2320	3.6-.7-.3	.71	1.16	.61	partial ligature	4½ months
P.L.VII	1755	3.8-.8-.3	1.01	.88	1.1	partial ligature	5½ months
P.L.I	2290	5-.8-.3	.85	1.14	.75	partial ligature	6 months
P.L.VI.B	1750	5-.8-.35	1.21	.88	1.38	partial ligature	6 months
P.L.V	1640	3.8-.8-.3	.92	.82	1.1	ligature of left branch	6 months
P.O.I	935	5.5-.9-.3	1.3	.44	3.0	clipping	14 days venous infarction
P.O.II	1815	4.5-.6-.25	.65	.91	.71	clipping	14 days
P.O.III	545	3-.8-.3	.51	.28	1.8	clipping	1 month

Spleen of Control Rats.

Animal No.	Body weight in gm.	Size of spleen in c.m.	Spleen weight in gm.	Estimated normal weight
N.1.	165	3.1-.6-.3	.45	.44
N.2.	195	3.1-.7-.3	.46	.52
N.3.	210	3.2-.8-.3	.64	.57(pregnant)
N.4.	185	3.1-.6-.3	.43	.50
N.5.	150	3.1-.7-.2	.44	.40
N.6.	185	3.2-.7-.3	.51	.50
N.7.	210	3.2-.7-.3	.54	.57
N.8.	180	3.2-.7-.3	.58	.49

The normal weights have been estimated on the ratio of .27% body weight (Jackson, 1915; Hatai, 1913).

Spleen of Control Rabbits.

Animal No.	Body weight in gm.	Size of spleen in c.m.	Spleen weight in gm.	Estimated normal weight
N.1.	900	3.2-.7-.2	.42	.45
N.2.	940	3.2-.6-.2	.39	.47
N.3.	1150	3.4-.8-.2	.58	.57
N.4.	950	3.1-.7-.25	.45	.48
N.5.	1250	3.2-.8-.2	.54	.62
N.6.	910	3.2-.7-.2	.48	.45
N.7.	1650	4.5-.8-.2	.74	.82
N.8.	850	3-.6-.2	.38	.43

It will be noticed that the control weights are slightly less than the estimated normal weights on the basis of .5 gm per kilo the maximum in Krumbhar's figures (Krumbhar, 1926).

few islands of lymphoid tissue lying in a mass of blood which had not however coagulated, but had retained to a great extent the configuration of the spleen owing to the presence of a thinned out lace work of reticular mesh (see Fig.1). The sinuses, trabecular veins and pulp veins could not be distinguished except by special staining. At the extreme periphery of the lobule the sinuses then appeared as distended sacculi.

In rats and rabbits dying in 24 to 48 hours after operation the congestive reaction was still marked. The engorgement was distinctly subcapsular and peri-trabecular, forming a distinct zone involving the periphery of the splenic lobule leaving the malpighian follicles comparatively free. They were however com-:pressed by the distension of blood beyond the marginal zone, where the arterial capillaries open out into the reticular mesh. In places, a few red blood cells had percolated into the follicles. The secondary lymphoid foci in the rats' spleen appeared compressed and grouped together by the congestion of the pulp. The reticular syncytium was distinct, but the mesh work was irregular and appeared broken up and the number of nuclei reduced. Here and there haemorrhages had taken place.

The Effect of Partial Obstruction.

In rats after partial obstruction of two to four and a half months duration, induced by an elastic ligature/

ligature, the spleen had undergone considerable shrinkage as compared with the acute stages of congestion as seen at laparotomy one week after operation. The capsule was slightly more opaque than normal and the whole organ had a lobulated appearance. Microscopically congestive changes were quite well defined. Sinus engorgement was most marked in the subcapsular and peritrabecular zones. In the perimalpighian zone there was a diffuse engorgement of the pulp mesh. The capsule and trabeculae were thickened and had caused irregular contraction of the organ. The malpighian follicles appeared unaffected except for a slight increase in the reticular mesh. With azan staining there was slight fibrillary increase in the pulp.

In rabbits killed from 4½ to 6 months after operation there was little splenic enlargement (see protocol II). The capsule was generally thickened and more opaque than normal. In some spleens, an irregular lobulation was seen on the surface. Histologically, venous congestion was indicated by dilatation of the pulp veins and the sinuses at the periphery of the lobule (see Fig.2 and Fig.5). The trabecular veins were markedly engorged. There was an increase in the fibrillary reticulum in the pulp around the dilated sinuses and pulp veins. Thickening of the capsule/

capsule and trabeculae was quite distinct, and spread of fibrils from the trabeculae into the surrounding pulp would occasionally be made out. With regard to the malpighian follicles, they were variable in size; some had undergone atrophy while others were unaffected. An increase of the lymphoid reticulum of the follicle could be made out, but this seemed to be related to a slight general increase in the fibrillary mesh work rather than any definite spread from the arterioles. On the whole the appearance was suggestive of a fibroid atrophy which was compensated by the state of distension of the sinuses. Proliferative changes were absent in the pulp cords or in the malpighian follicles. The appearance of lobulation was due to the contraction of the trabeculae.

In rabbits after partial obstruction had been induced by clipping, one (rabbit P.O.I) developed thrombosis of the portal vein and died two weeks after the operation. The condition of the spleen was one of venous infarction. In the others the congestive reaction was quite marked two weeks and one month after operation, indicating that some degree of portal stasis had developed. The histological changes were more or less similar to those induced by partial obstruction by ligature, but capsulo-trabecular thickening and fibrosis had not commenced.

In/

In two rats an attempt was made to induce partial obstruction by passing a loop round the portal vein and tying it up to the under surface of the liver. However, both animals developed portal thrombosis and died from haemorrhage into the intestine. The condition of the spleen was one of venous infarction.

The changes in the liver in rats and rabbits were not constant. Twenty-four hours after obstruction had been induced by ligature, the only histological change that was noticeable was slight swelling of the cytoplasm of the liver cells and a paler staining reaction. Necrotic changes were not present. In cases where chronic obstruction had been induced in rabbits by partial ligature for 5½ to 6 months, the liver showed slightly hyalinised tissue in the portal tracts. The bile ducts appeared thickened and slight lymphocytic infiltration was present around the portal tracts. Some of the Küpffer cells appeared swollen and distended with pigment. The appearances suggested a slight degree of collapse sclerosis around the portal vein. The liver cells appeared healthy.

The Effect of Portal Phlebitis.

Following portal phlebitis induced by cauterising the vein with pure carbolic acid and a strong solution of iodine, it was found that four months after the operation the under surface of the liver and the omentum/

omentum had all become matted together to form a thick irregular mass in which the portal vessels were im-:bedded. There was little splenic enlargement. Histologically the spleen showed marked capsulo-trabecular thickening, engorgement of the sinuses, the pulp veins and the trabecular veins. The malpighian follicles showed little alteration. Fibrillary increase was little marked, nor were there any changes in the malpighian follicles suggestive of "fibro-adenie". In the liver there were well marked focal infiltrations round the portal tracts; mononuclear cells and lympho-:cytes had grouped themselves in thick clusters around the portal venules and spread irregularly along the sinusoids; a few polymorphonuclear leucocytes were intermingled and the histological picture was that of a diffuse hepatitis spreading from the portal tracts (see Fig.6). There was little destruction of tissue or fibrosis. The epithelium lining the bile ducts appeared more active than normal. Here and there around the portal tracts collections of haemosiderin could be made out. The hepatic cells showed in places slight hydropic changes and in other areas there were large nuclei suggestive of regeneration. The whole series of changes appeared diffuse except for a more localised damage round the portal tracts.

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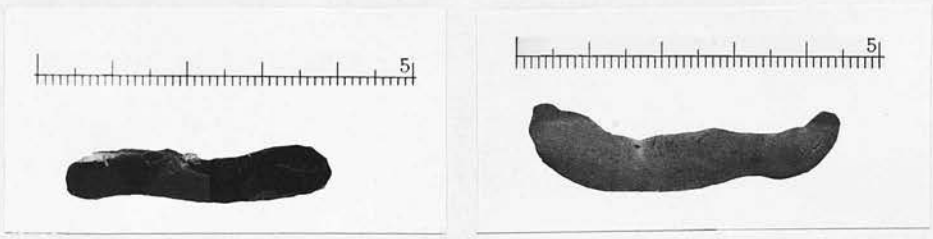
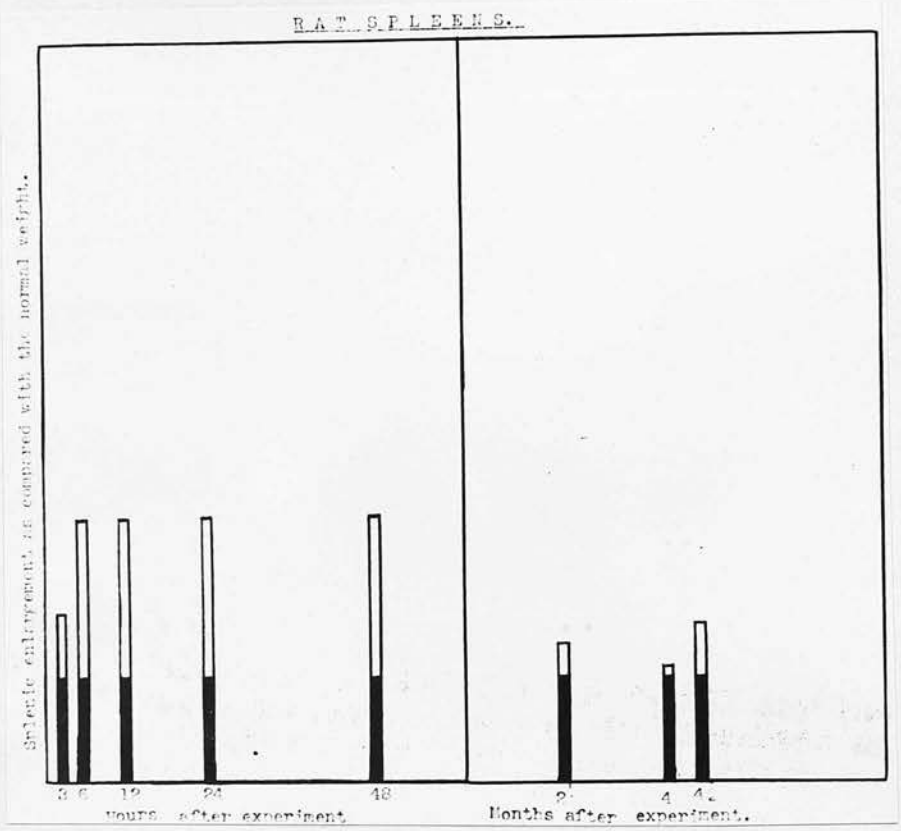
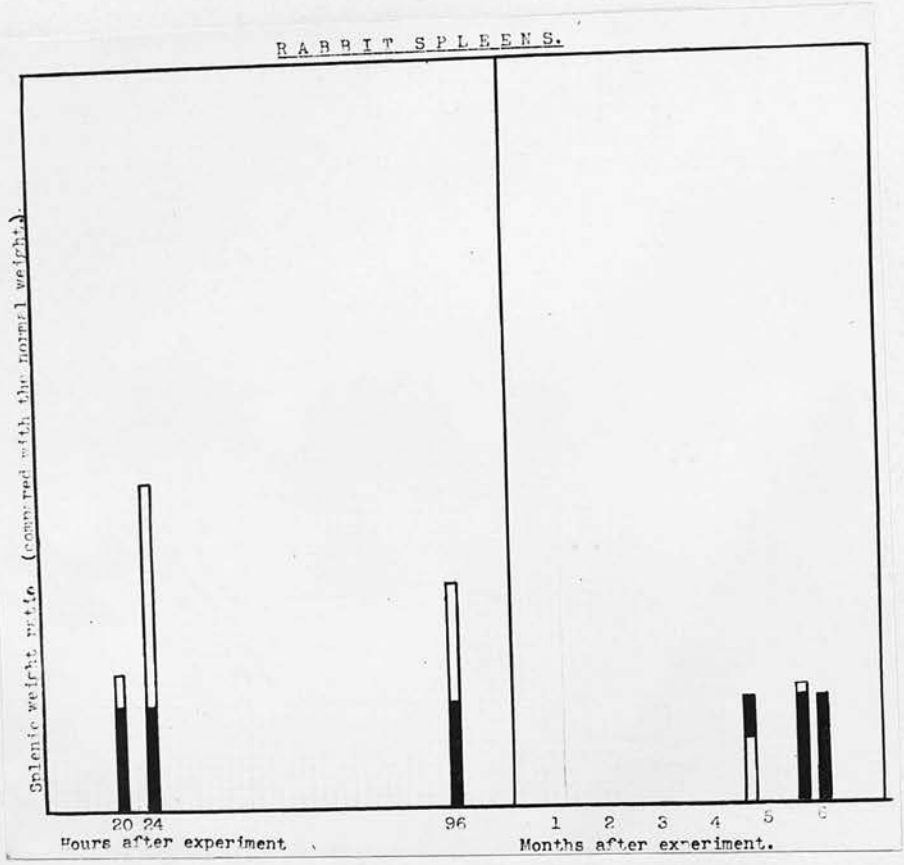


Spleen of rabbit P.L.VI four and a half months after partial obstruction to the portal vein. Note the thickening of the trabeculae and the absence of any splenomegaly as compared with the spleen of a healthy rabbit weighing 1450 gm. on the right.



Histogram I.

Rats' spleens showing the effect of complete portal obstruction in inducing an enlargement due to extreme congestion, as compared with the effect of chronic portal obstruction. The black columns show the estimated weights of the normal spleen.



Histogram II.

Rabbits' spleens showing the congestive enlargement due to complete portal obstruction and the relatively slight enlargement in chronic portal obstruction.

The Effect of Obstruction to the Left Main Branch of the Portal Vein.

With complete ligation of the main left branch close to the under surface of the liver 4½ months after the operation the spleen of a rabbit (P.L.V) showed little increase in size. The surface was slightly irregular and the capsule somewhat opaque. Histologically there was moderate congestion; the trabeculae and capsule appeared a little thicker than normal. The appearance of the liver was more or less similar to what has been described by Rous and Larrimore (1920). The right anterior, the left anterior and posterior lobes which constitute the main liver mass had undergone extreme atrophy. The liver mass was white, firm and rubbery in consistence, and on section the appearance was not unlike that of pancreatic tissue. Very little soft liver tissue could be made out; there was no suggestion of a coarse cirrhosis with irregular nodules and scarring in between. The right posterior and the caudate lobes had undergone extreme hypertrophy and extended down almost to the brim of the pelvis on the right side along the dorso lateral aspect of the abdomen. This hypertrophied mass corresponded more or less to the left segment of the liver; it was dark purple in colour while the capsule was tense and shiney. Histologically the atrophied left segment of the/

the liver showed a few small rounded islands of liver tissue. Some of these were small round clusters of three or four liver cells, others were larger lobuli where the liver cells had lost their normal arrangement and had formed rounded masses with little sinusoidal tissue in between. Some of the cells showed hydropic degeneration. Around these islands, numerous bile ductules appeared lying in a stroma which was in great part composed of the collapsed framework of the liver (see Fig.3). There was however some periductal increase of fibrous tissue and proliferation of the bile ducts. The portal venules appeared as elongated fibrous cords with collapsed and obliterated lumina. The hepatic arterioles appeared comparatively smaller than normal. The hypertrophied right segment of the liver showed a more or less normal structure (see Fig.4).

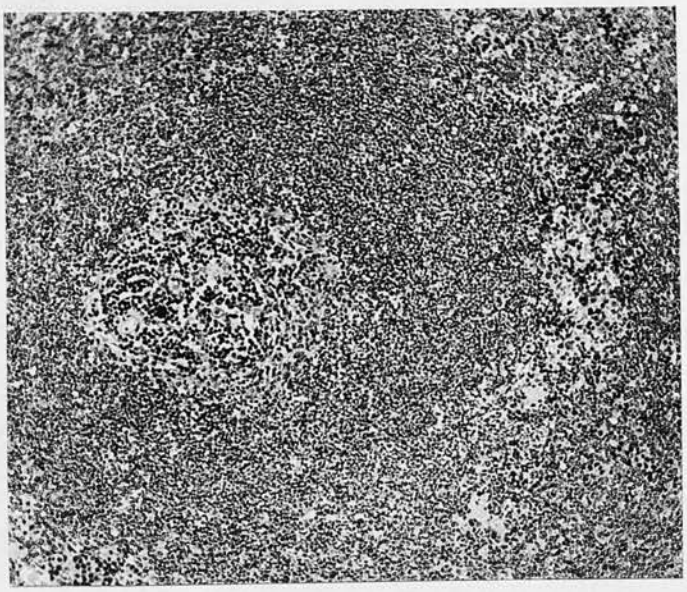


Fig.1. Rat spleen (P.L.VII) 12 hours after complete ligature of the portal vein. The appearance is one of venous infarction; the whole pulp is a mass of blood with little syncytial mesh visible. The malpighian follicles and focal lymphoid clusters are compressed and isolated. (x 120) H. & E.

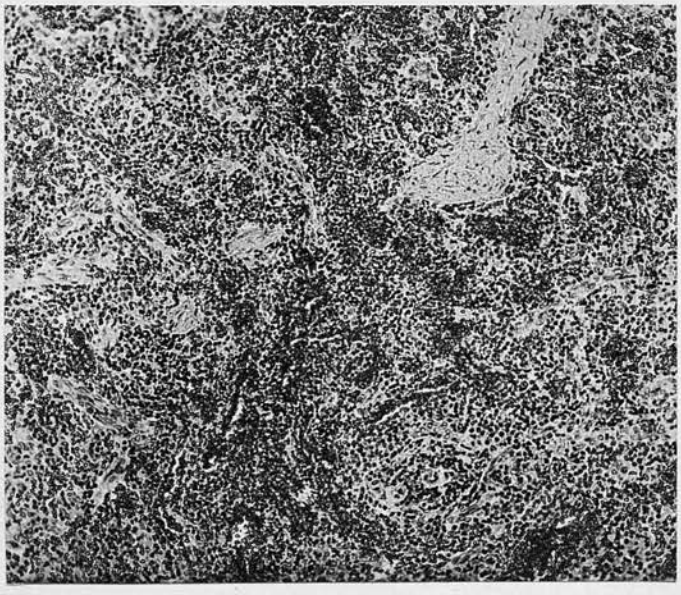


Fig.2. Rabbit spleen (P.L.VI) 6 months after partial ligation of the portal vein. Note the congestion of the sinuses and pulp veins, the haemorrhages and trabecular thickening. (x 120) H. & E.

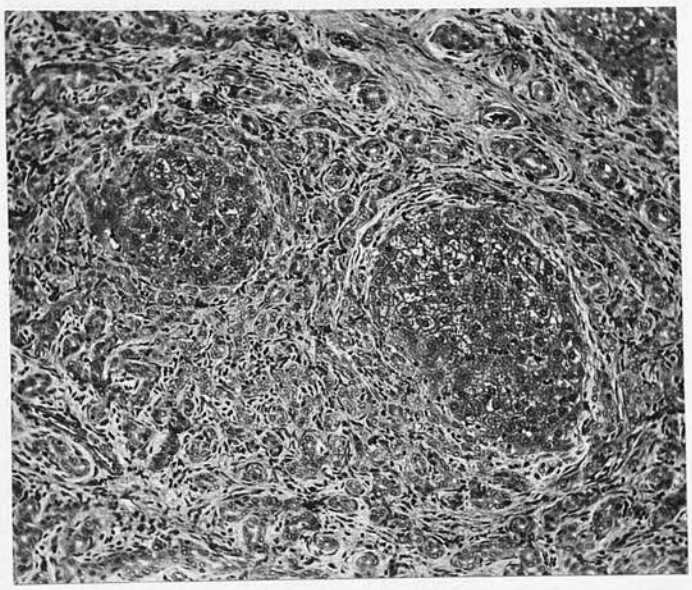


Fig.3. Rabbit liver (P.L.V) 6 months after ligation of the main left branch of the portal vein. The left lobe showing remnants of lobules surrounded by the condensed stroma. There is some bile duct proliferation. The liver cells show hydropic change. (x 120) H. & E.

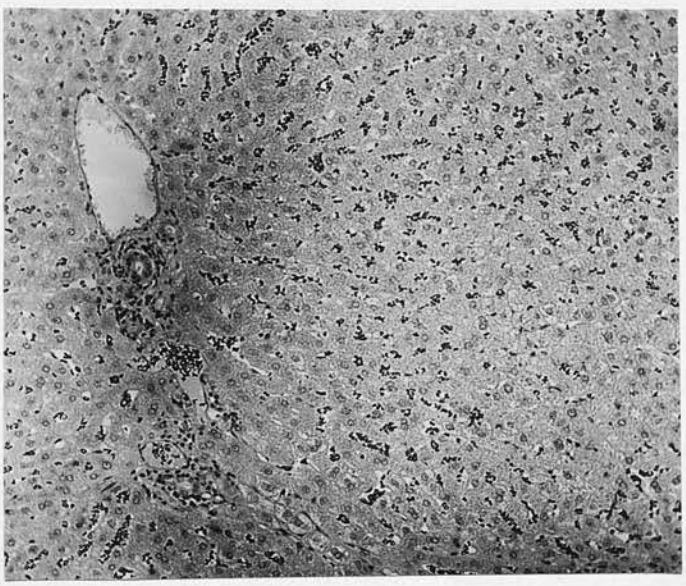


Fig.4. Rabbit liver (P.L.V) the hypertrophied right lobe 6 months after ligation of the main left branch shown for comparison with the left lobe above. (x 120) H. & E.

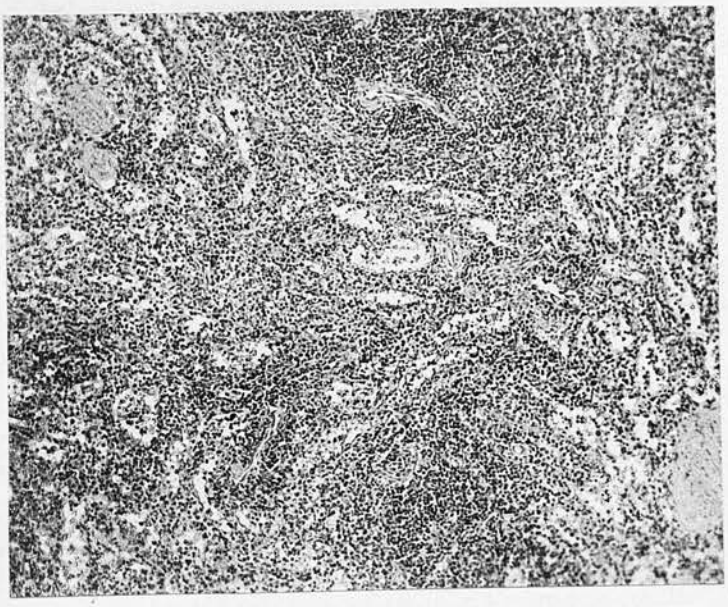


Fig.5. Rabbit spleen (P.L.VI.b) showing dilatation of the sinuses after chronic congestion of 6 months duration (x 95). Anderson's haematoxylin and Van Gieson.

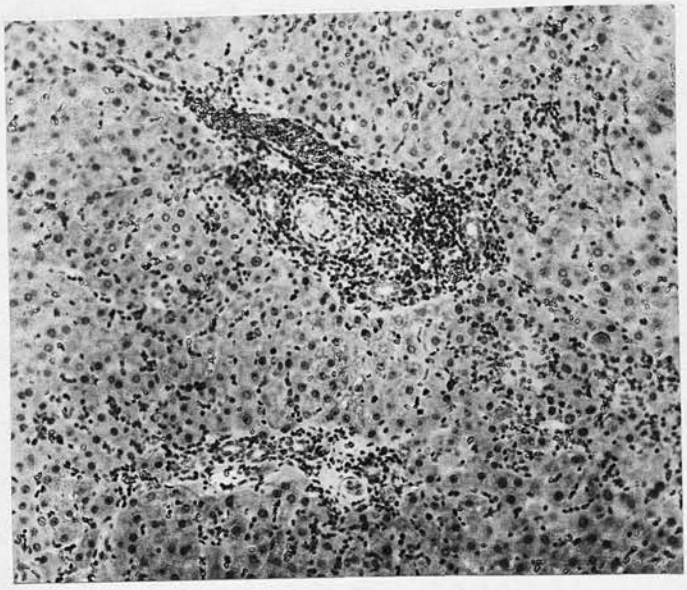


Fig.6. Rat liver (PP.II) four months after portal phlebitis. Note the focal infiltration round the portal tracts and the diffuse hepatitis. (x 120). H. & E.

DISCUSSION.

Histological studies on the condition of the veins in the spleen have shown that following these different methods of inducing portal obstruction there is an engorgement that is kept up for as long as the obstruc-:tion is maintained. The effect of acute obstruction in causing diffuse haemorrhage and producing venous infarction of the spleen had demonstrated that a sudden and marked increase in the portal pressure will induce splenic enlargement of two or three times the normal and no more. It has also been shown that narrowing of the lumen, by a small fraction by the method of clipping, or to the extent of two thirds to half the diameter by ligation of the vein, is followed by a less marked but more chronic congestive reaction that should correspond to a condition of phlebosclerosis in man. This chronic stasis shows many features of resemblance to that of cardiac congestion in man, though Jäeger (1931), Hueck (1929) and Klemperer (1936) regard the histological changes as different. The gradual thickening and fibrosis of the capsulo-trabe-:cular system, the increase in the fibrillary reticulum, the distension and gradual dilatation and rigidity of the sinus walls are also similar to the changes met with in other organs such as the liver and the lung in/

in cyanotic induration. In chronic venous congestion in man Sokoloff (1888) has described the changes in the spleen as consisting of (1) a dilatation and distension of the venous sinuses with a gradual thickening of the adventitial lining without any changes affecting the endothelium; (2) a thickening of the supporting tissue which also extends to the reticulum of the malpighian follicles and the adventitial sheaths of the vessels, and (3) a slight deposit of haematogenous pigment in the pulp. It will be seen that the histopathological changes in rats and rabbits following partial ligature are almost identical.

There is little resemblance of the changes in the spleen to the picture of the splenic anaemia syndrome in man even though fibrosis and fibrillary increase are met with. It is the proliferative reaction with fibrillary increase that Gauckler (1905) described as a "sclerose hypertrophique pulpaire" that characterises splenomegalic cirrhosis. The post congestive indura-:tion and fibrosis resembles more the "sclerose atrophique" reaction and ends in cyanotic atrophy rather than hypertrophy. Hueck (1929) has argued that in peripheral stasis as in obstruction to the splenic and portal veins the effect of the collateral circula-:tion would be to render the congestion intermittant and cause hypertrophic changes. The experimental work/

work of Wicklein (1891) and Warthin (1911) by obstruc-:tion to the splenic veins as well as the present study of the effects of portal obstruction tend to show that venous congestion in the spleen of whatever type is followed by an immediate and progressive enlargement which lasts only for a short time and is gradually followed by shrinkage and fibrosis. Hyper-:plastic reactions do not play any part in the experi-:mental animal after portal obstruction. On the other hand the later shrinkage of the spleen seems to be more related to the gradual atrophy of the pulp from the compression of the syncytium by the distended vessels and the erythrocyte accumulations in the pulp mesh. A gradual fibrillary increase would also favour this shrinkage while the turgidity of the veins and sinuses and the rigidity of their walls would tend to counter-:act this factor. These experiments therefore do not support the view that the gross splenomegaly and hyper-:plastic changes which characterise the Banti syndrome are due to a mechanical block in the portal circulation. Some other factor is essential to induce proliferative changes in the spleen.

SUMMARY.

1. It has been possible to induce portal obstruction in rats and rabbits by various operative measures of narrowing the lumen of the portal vein.

2. After complete occlusion of the vein there is a splenic enlargement of twice or three times the normal and no more; the condition of the spleen is one of venous infarction.

3. After partial obstruction of 3 to 6 months duration there is very little splenic enlargement in rats and rabbits. The spleen shows dilatation of the sinuses, distension of the pulp veins and trabecular veins and a variable atrophy of the pulp with slight fibrillary increase. Hyperplastic reactions are absent.

4. These experiments do not support, but seem to negativate the view that the Banti syndrome is due to a blockage of the portal vein.

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PATHOLOGICAL STUDIES ON SPLENOMEGALY.

PART I. EXPERIMENTAL STUDIES.

CHAPTER II.

THE SPLENIC REACTION IN EXPERIMENTAL CIRRHOSIS AND PRE-CIRRHOTIC TOXAEMIAS: THE PATHOGENESIS OF AN EXPERIMENTAL BANTI LESION.

THE SPLENIC REACTION IN EXPERIMENTAL CIRRHOSIS AND PRE-CIRRHOTIC TOXAEMIAS: THE PATHOGENESIS OF AN EXPERIMENTAL BANTI LESION.

INTRODUCTION.

The behaviour of the spleen in human cirrhosis has been variously interpreted. The splenomegaly has been looked upon as a secondary effect of the hepatic lesion, as a simultaneous toxic effect of the same poison that caused the hepatic lesion, and again as an independent splenic disorder that brought about the liver cirrhosis. The correct mode of approach to settle this question was to study the effects of well known cirrhogenic agents by experimental methods. In the varying stages of experimental cirrhosis it is possible to grade the effect on the spleen of the hepatic damage and of the liver poison "per se". The possibility of the production of hepatic lesions by a hypothetical spleno-toxin is also a subject which has to be considered.

Scope of the Investigation.

In order to induce varying grades of toxic damage and varying lesions, it was decided to work with toxic agents which had been previously used to produce ex-:perimental liver necrosis and cirrhosis. Thus the work of Findlay (1924) and of Hurst and Hurst (1926) have established that manganese chloride has the effect of/

of producing a monolobular cirrhosis in rabbits and guineapigs. Gardner and his co-workers (1925) have shown that carbon tetrachloride produces central necrosis and multilobular cirrhosis in animals. Similarly Davidson (1935) working with retrorsine, an alkaloid obtained from ragwort, Senecio retrorsus, has found that it acts as an endothelial toxin on the liver inducing haemorrhage, necrosis and cirrhosis. Though little evidence of splenic damage has been recorded in these experiments, it was thought that this was because the hepatic lesions were in the forefront and easily recognized while evidence of splenic damage would require special study. A histopathological study of the splenic reaction during the various stages of hepatic damage has the advantage that one could grade the lesion from an early or progressive toxic effect to a late effect following the development of cirrhosis.

I. EXPERIMENTAL MANGANESE TOXAEMIA.

Review of Literature.

The effects of manganese poisoning were first described in man, but attracted little attention even though cases were reported from time to time (Couper, 1837; Friedel, 1903; Van Jaksch, 1913; Casamajor, 1913). The occurrence of Parkinsonism in men working in an atmosphere of manganese dust described by Edsell, Wilbur and Drinker (1919) focussed interest on the nervous system in view of the similarity of the symptoms to encephalitis lethargica and hepato-lenticular de-:generation. Cases subsequently reported described mostly the nervous manifestations of chronic manganism (Davis and Huey, 1921; Charles, 1922; Flintzer, 1931; Mosheim, 1932; Baader, 1932; Salmon and Planque, 1933). In autopsy records, Casamajor (1916), Gayle (1925), Ashizawa (1927) and Canavan, Cobb and Drinker (1934) do not mention any significant splenic changes.

In experimental work with the demonstration of changes in the corpus striatum by Lewy and Tiefenbach (1921) interest was centered on the nervous system. However, Lund, Shaw and Drinker (1921) found a hepatic localisation after intravenous injections of manganese dioxide and the possibility of visceral lesions was brought out by the experiments of Drinker, Shaw and Drinker (1923) who produced fibrosis of the liver in cats/

cats after large single doses of manganese. Findlay (1924) produced a well marked monolobular cirrhosis in rabbits and guineapigs by injections of manganese chloride. He suggested that the effect was due to the excretion of manganese through the biliary passages. Mella (1924) in his experiments on monkeys had at the same time found degenerative changes in the globus pallidus as well as in the liver. These results were confirmed by Handovsky, Schulz and Staemmler (1925) in their work on rabbits. Further studies by Hurst and Hurst (1928) brought to light the stages of the development of the liver necrosis and subsequent cirrhosis, though they could not define any nervous lesions. In subsequent work, Grünstein and Popowa (1929), Pugnatori (1932), Matsumara (1933) and Hendrych and Kliniesch (1935) have demonstrated that diffuse degenerative changes are actually found in the brain. Further, Grünstein and Popowa (1929) described that the small cell systems of the caudate nucleus are particularly involved. The difference between the hepatic lesions in the acute and the chronic manganese toxaemia is stressed by Rao (1931).

Though in these later experiments visceral changes other than liver necrosis are mentioned, little work has been done to study the behaviour of the spleen. However, scattered references to splenic changes are present/

present. Findlay (1924) described the spleen as generally enlarged and congested in experimental rabbits and Handovsky, Schulz and Staemmler (1925) have noted congestion of the sinuses and erythrophago-:cytosis by the pulp cells. Grünstein and Popowa (1929) have described hyperplastic changes in the malpighian follicles and pulp cords.

Material and Methods.

40 rabbits averaging about 900 gm. in weight were used for these experiments; 8 others were kept as a control series. Manganese chloride dissolved in normal saline in strengths varying from 0.5% to 1% of the hydrated salt was injected subcutaneously in one group and intramuscularly in another. For intra-:venous injections 0.25% solution in saline was used. The injections were given twice weekly with the larger doses (15-20 mg.), and thrice weekly with the smaller doses (7-10 mg. per kilo weight). When large single doses were administered the animals were allowed to die of the toxic effect, but with repeated doses they were killed at intervals by dislocation of the neck. At autopsy, the entire spleen was removed, weighed and measured before fixation; the greatest length, breadth and thickness were recorded as these gave a relative idea of the extent of the enlargement; the liver was examined/

examined in every case; of the other organs, the kidneys and the heart muscle were frequently examined; an examination of the nervous system was outside the scope of this investigation. The tissues were mostly fixed in Helly's fluid, while for frozen sections pieces of the spleen fixed in 10% formol saline. Paraffin sections were mounted on albuminised slides and stained by the following methods: (1) Mayer' s haemalum and eosin; (2) Leishman's stain for tissues as described in the appendix; (3) Heidenhain's azan stain; (4) Wilder's modification of the Foot-Biels-:chowsky stain for the reticulum; (5) Anderson's iron haematoxylin and Van Gieson's stain; (6) Perl's Prussian blue reaction.

General Effects of Manganese Toxaemia.

Progressive loss of weight was noticed in all the animals after repeated administration of manganese. In young animals there was considerable shock following the injections as shown by prostration and torpor. Many refused to take food and gradually passed into a condition of coma. In two animals paralysis of the hind legs was noticed and this was followed by a gradual weakness of the front legs. Jaundice was not met with, but the urine was high coloured. When solutions stronger than 0.75% were used there was considerable local reaction, but if the needle was passed/

passed in different directions and the fluid spread out by massage local ulceration could be prevented. As a rule the intravenous injections were well tolerated. With intramuscular injections it was found essential to change the site of injection frequently to avoid ulceration. On the whole, subcutaneous injections were easiest, provided care was taken to utilise different areas of skin so as to allow for local absorption.

Coccidiosis was met with as a complication in two animals of this series. These have been excluded from the series for the sake of purity of the results even though Guy and Purdy (1922) have remarked that the lesions of coccidiosis are too localised and dis-:tinctive to interfere with the histological results.

The Effect of Single Toxic Doses.

Following injections of 60 mg. of manganese chloride per kilo weight death occurred in two animals in 18 and 48 hours. In 18 hours the spleen showed little macroscopic change. Histologically the chief changes were in the syncytial nuclei of the pulp and at the marginal zone of the malpighian follicles. A thickening and irregularity of the nuclear membrane was distinct. The cytoplasmic syncytium appeared to have undergone a marked swelling so that the pulp mesh was gradually reduced to the form of vacuolar spaces. The/

Protocol of animal experiments. I.

A. Rabbits. Acute Manganese Toxaemia with large doses.

Ani-:mal No.	No. of injec-:tions	Body Wt.in gm.	Mgm. per kilo. body wt.	Size of spleen in cm.	Spleen Wt. in gm.	Estimated normal weight.	Wt.Ratio (enlarge-:ment).
12.	1	624	10	3-0.3-0.25	0.22	0.31	0.7
11.	1	910	11	3-0.25-0.25	0.22	0.45	0.5
78.	1	680	60	3-0.5-0.2	0.39	0.34	1.1
96.	1	910	60	4-0.6-0.3	0.78	0.46	1.7
B. Repeated toxic doses of Manganese Chloride.							
514	2	618	15	3-0.6-0.3	0.33	0.31	1.1
707a	4	559	70	2-0.5-0.2	0.29	0.28	1.0
512	12	553	320	4-0.6-0.3	0.32	0.28	1.1
707b	12	480	330	3-0.6-0.3	0.25	0.24	1.0
511	31	960	490	4-0.6-0.35	0.83	0.48	1.7
509	31	900	520	4-0.8-0.25	0.98	0.45	2.2
310	56	1580	575	5.3-1.4-0.35	1.85	0.79	2.3
508	53	1443	585	5.5-1.0-0.3	1.46	0.72	2.0
706	51	1248	645	4.6-0.9-0.35	1.68	0.62	2.7
513	58	950	990	5.0-1.0-0.3	1.05	0.48	2.2
C. Repeated small doses of Manganese Chloride.							
20	4	1000	25	4.0-0.5-0.2	0.65	0.5	1.3
44	4	930	45	4.1-0.7-0.3	0.83	0.47	1.8
45	4	910	55	4.0-0.7-0.3	0.81	0.46	1.8
46	4	960	55	3.8-1.0-0.3	0.81	0.48	1.7
79	4	700	65	3.9-0.9-0.3	0.78	0.35	2.2

C. (continued).

Ani-mal No.	No. of injec-tions	Body Wt. in gm.	Mgm. per kilo. body wt.	Size of spleen in cm.	Spleen Wt. in gm.	Estimated normal weight.	Wt. Ratio (enlarge-ment).
15	7	1360	65	7.5-1.0-0.2	1.36	0.68	2.0
25	12	700	140	3.0-0.8-0.2	0.57	0.35	1.6
24	16	1236	165	5.0-1.2-0.3	0.95	0.62	1.5
23	19	1230	185	6.0-0.8-0.2	1.05	0.62	1.7
18	21	1230	205	4.5-1.1-0.3	1.18	0.62	1.9
28	27	1360	250	5.0-1.2-0.6	2.65	0.72	3.7
30	18	650	270	3.0-0.5-0.2	0.39	0.32	1.2
26	21	810	290	4.1-1.0-0.25	0.95	0.41	2.3
32	33	1250	360	5.8-0.6-0.2	0.97	0.62	1.6
510	36	1455	370	5.0-0.9-0.35	1.6	0.72	2.2
14	30	1020	390	3.6-0.9-0.35	1.65	0.51	3.2
22	37	1250	415	5.8-1.1-0.35	2.08	0.62	3.4
+29	45	1930	320	5.6-1.5-0.35	2.25	0.97	2.3
+16	45	1870	335	5.0-1.2-0.3	1.8	0.93	1.9
+19	44	1755	340	3.8-1.2-0.5	1.89	0.88	2.1
+17	45	1640	375	6.1-1.0-0.3	1.95	0.82	2.4
+27	44	1475	400	5.0-0.8-0.35	1.65	0.74	2.2
++31	24	670	425	3.8-0.8-0.25	0.75	0.31	2.1

+Injection of very small doses of 7 mg. per kilo wt.

++Injection of varying doses.

Not included: No.0. Complicating sepsis.
No.13 and No.42. Coccidiosis.

Control Rabbits.

Animal No.	Body Weight.	Size of Spleen.	Spleen Weight.	Estimated Normal Weight.
N.1	900	3.2-0.7-0.2	0.42	0.45
N.2	940	3.2-0.6-0.2	0.39	0.47
N.3	1150	3.4-0.8-0.2	0.58	0.57
N.4	950	3.1-0.7-0.25	0.45	0.48
N.5	1250	3.2-0.8-0.2	0.54	0.62
N.6	910	3.2-0.7-0.2	0.48	0.45
N.7	1650	4.5-0.8-0.2	0.74	0.82
N.8	850	3.0-0.6-0.2	0.38	0.43

It will be noticed that the control weights are slightly less than the estimated normal on the basis of 0.5 gm. per kilo., the maximum in Krumbhaar's figures (Krumbhaar, 1926).

The change seemed more or less an exaggeration of the softening of the cytoplasm of the pulp syncytium which Hueck (1928) has described as an alteration during the normal relaxation of the spleen (see diagram II). Helmke (1935) refers to a similar condition in the human spleen as parenchymatous oedema, but here in the rabbit the condition was more similar to cloudy swell-:ing of the syncytial cytoplasm rather than simple oedema. In places, acidophilic staining was marked, indicating the beginning of coagulation necrosis. The free cells in the pulp were not so much affected. Similar changes were present in the marginal zone of the follicle where occasionally frank peripheral necrosis of the syncytium and of the lymphoid cells were present. In 48 hours the changes were well marked and the whole syncytium appeared eosinophilic and the meshwork of the pulp became indistinct. The necrotic changes had affected some of the nuclei of the pulp and many of the lymphocytes showed karyorrhexis.

In the liver the hepatic cells showed an alteration in the staining reaction owing to the formation of innumerable small globules only some of which gave the staining reactions of fat. Condensation and irregul-:arity of the nuclear membrane suggested damage to the nuclear apparatus. In places, around the bile ducts and the portal tracts, the liver cells took on a pale pink/

pink stain with eosin and the nuclear changes of necrosis were more definite. On staining with the Leishman stain the contrast between the necrotic eosino-:philic areas and the comparatively healthy liver cells which were more basophilic and granular, was well brought out. Even as early as 48 hours after injection the bile duct epithelium appeared to be active and dividing, forming pale-staining oval cells resembling endothelial cells. On the other hand the Küpffer cells lining the sinusoids had often pyknotic and irregular nuclei and there was no suggestion of any proliferative change. In 48 hours the necrotic reaction was quite distinct at the periphery of the lobule and a peri-ductal arrangement was quite notice-:able. Haemorrhages into the necrotic zones were exceptional, but a variable congestion of the sinusoids was present.

In the kidneys the epithelium of the convoluted tubules showed cloudy swelling; marked congestion and slight swelling of the glomeruli were present, but there was little suggestion of any marked toxic damage; no focal collections of inflammatory cells were present in the boundary zone though Grünstein and Popowa have described their occasional occurrence. Swelling of the endothelial cells of the tuft was marked in some cases.

Effect/

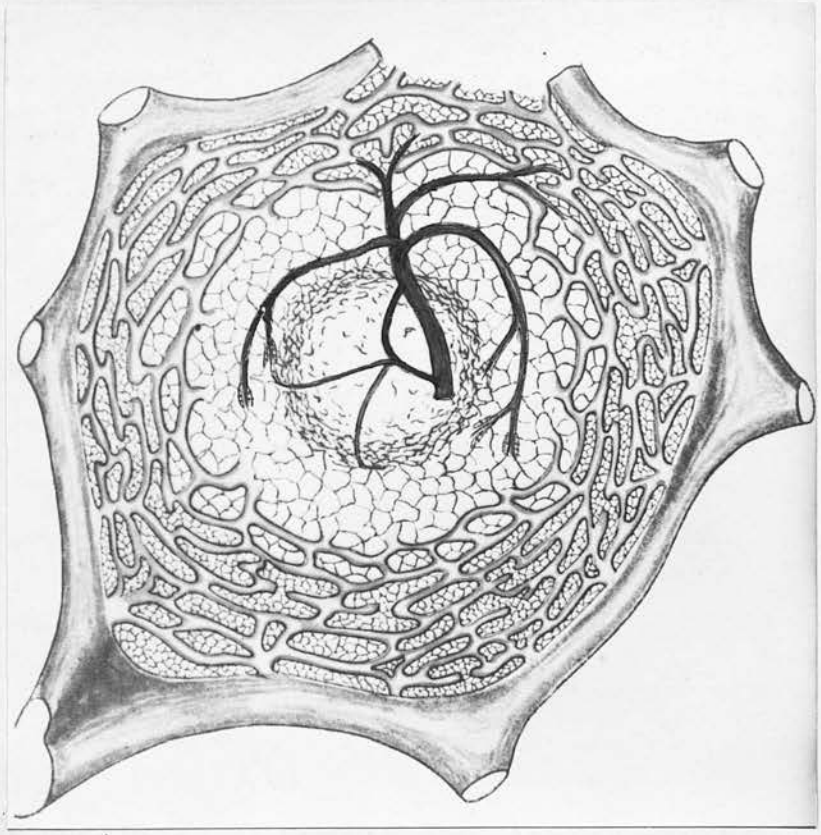


Diagram I (partly after Hueck) of the malpighian follicle showing the eccentric arteriole and its branches (red). Note the opening of the arterial twigs at the periphery of the follicle into close capillary mesh at the marginal zone, the opening of the circumflex branches through the ellipsoids into the pulp beyond the marginal zone, and the long vessels that open into the sinuses at the periphery of the splenic lobule. During venous congestion the system of sinuses would approach closer to the marginal zone.

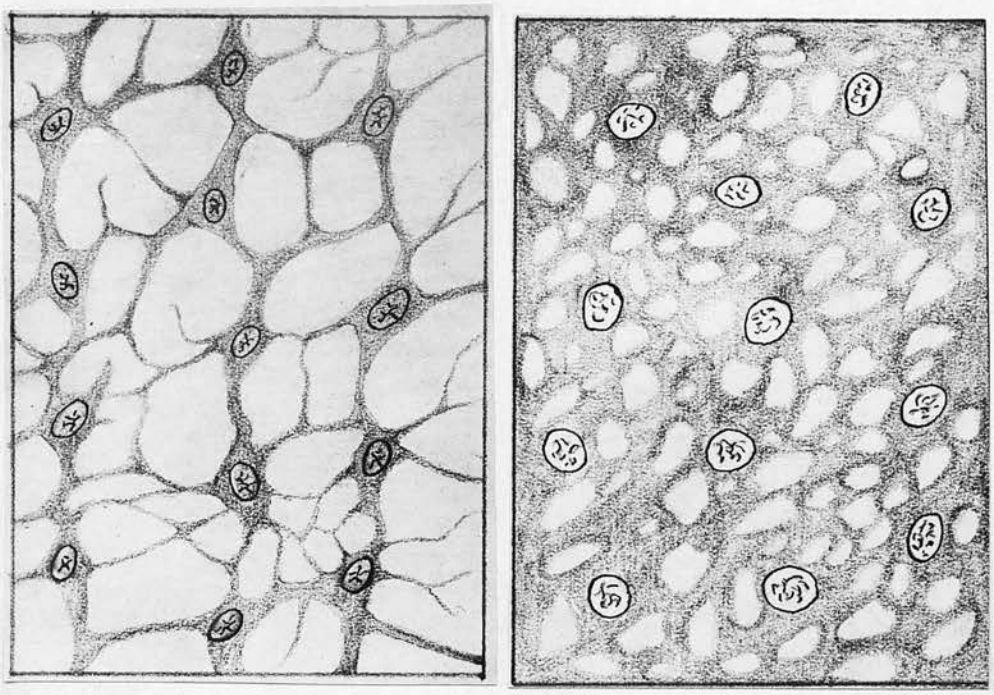


Diagram II (after Hueck) showing the reticulum of the pulp. It will be noticed that the pulp mesh is made up of a syncytium and without actual cell boundaries. This syncytium is called the "protoplasmic reticular syncytium" in contradistinction to another meshwork that follows closely the outlines of this cytoplasmic mesh. This meshwork is called the "fibrillary reticu-:lum" and is composed of argentophile fibrils which show branching and anastamosis (see Diagram III). The pulp syncytium may be thin and stretched or may show liquefactive softening as shown above. An exaggeration of the latter state is found in cloudy swelling and necrosis.

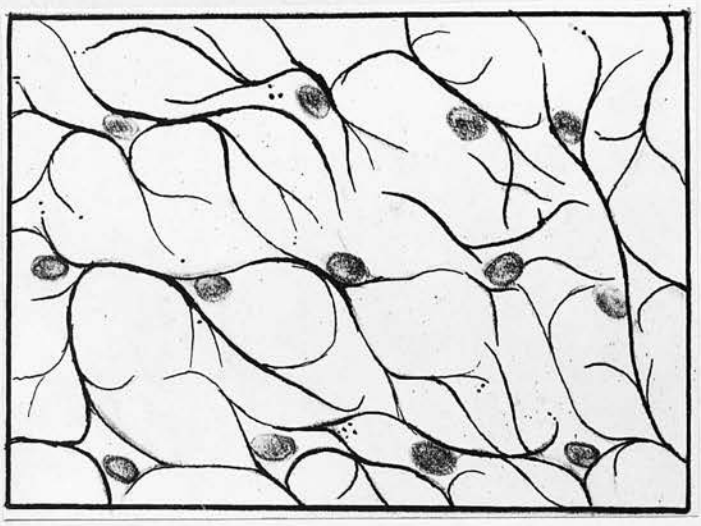


Diagram III showing the structure of the fibrillary reticulum which follows closely the branching of the protoplasmic reticular syncytium. The fibrils are argentophile. The nuclei shown are only faintly stained as they belong to the cytoplasmic mesh and are not the nuclei of the branching reticulin fibrils.

Effect of Repeated Toxic Doses.

Following 12 to 58 injections of 15 to 20 and 20 to 30 mg. of manganese chloride per kilo weight amounting to 330 to 990 mg. the changes met with were as follows:-

Macroscopically the spleen showed an increase in size and weight averaging about twice the normal (see histogram I). Little evidence of congestion could be judged by any alteration in the colour of the organ. The capsule was tense, dark greyish purple in colour and slightly more opaque than normal. The liver also appeared slightly enlarged with irregular pale mottling of the cut surface. With repeated injections of long duration, there was a slight increase in the consistence of the organ.

Microscopically the spleen showed changes in the malpighian follicles and an erythrophagocytic reaction in the pulp and the sinuses. Capsular thickening and fibrosis were generally present, but only to a slight extent. Most of the malpighian follicles were affec-:ted, but the change appeared more advanced in some than in others. In many cases a coagulation necrosis of the cytoplasmic syncytium of the marginal zone of the follicle was followed by a collapse of the mesh. A gradual increase in the argentophile reticulum fibres could also be made out. In the later stages, there seemed/

seemed to be a direct extension of fibrocytic tissue from the adventitial sheath of the follicular arterioles and their centripetal, and centrifugal branches. There was also evidence of a collagenous transformation of the condensed reticulum fibrils so that varying grades of peri-arterial fibrosis were produced. The changes were more marked at the periphery of the follicle where the eccentric artery curves in an arcuate fashion and sends circumflex branches while other branches end in the pulp at the periphery of the splenic lobule as the long penicillar arterioles. The peri-:arterial reaction was equally marked around the peni-:cilli ending in the marginal zone as well as in the longer twigs that passed through the pulp, (see diagram of the circulation of the malpighian follicle). Side by side with condensation of the syncytium and fibrocytic spread from the vessels, foci of karyolysis and pyknosis were present in the lymphocytes surround-:ing the vessel wall. The sparsely distributed nuclei of the lymphoid reticulum and of the syncytial mesh at the marginal zone showed similar necrobiotic changes in areas which had not undergone fibrosis. The appearance was typical of repeated necrotic damage and fibrous replacement. The endothelial lining of the arterioles and arterial capillaries was generally swollen in the earlier stages of the toxic process. The nuclei of these/

these cells appeared condensed and protruding into the lumen. In some follicles strands of condensed reti-:culum seemed to break up the nodule so that the lym-:phoid pattern was gradually lost. Congestion of the marginal zone was generally slight and haemorrhages were rare in this series.

The erythrophagocytic reaction was another striking feature that was almost constant. There was the gra-:dual mobilisation of large macrophage cells by a pro-:cess of differentiation of the littoral cells and the pulp syncytium. In many cases the sinuses at the periphery of the splenic lobule were filled with so many of these cells that their lumen appeared distended. Varying grades of erythrophagocytic activity were shown as in the studies of Addison (1919) where foreign red corpuscles were ingested. The mononuclear cells in-:gested entire red blood cells, some of which were dis-:tinctly haemoglobinous, others showing various stages of fragmentation and still others much smaller in size where from absorption free haemosiderin was formed. In many cases, the whole process of intracellular digestion and formation of haemosiderin could be traced. Large erythrophagocytes 20-24 μ in diameter were quite common, and occasionally giant forms 40-50 μ in size were met with; the cytoplasm of these cells contained as many as 20 to 24 ingested cells and often the inges-:ted/

:ted mass had fused together, so that the nucleus was obscured. All these cells gave a deep blue iron reaction. Besides red corpuscles, leucocytes and basophilic cells were also ingested and showed various stages of fragmentation and absorption. Occasionally the enlarged cell appeared necrotic; in smaller cells the nucleus was visible as a round or oval pale ring with an indistinct chromatin net work, but a clear nuclear membrane; sometimes the nucleus was completely obscured by the ingested material. Free mononuclear cells without pigment were also quite numerous and all the stages from the beginning of ingestive activity could be traced.

In the liver the hepatic cells showed well defined small areas of necrosis around the biliary tracts; sometimes the areas were larger and formed a definite zone of peripheral necrosis around the lobules (see Fig.3). The necrotic cells were fused together and formed granular eosinophilic masses in which some nuclear debris could be made out. The bile ducts showed well marked inflammatory changes. Groups of mononuclear leucocytes and lymphoid cells and a few polymorphs had formed irregular clusters around many of the bile ducts (see Fig.4). In many cases the cells had invaded the fibrous and submucous coats and the duct epithelium. The epithelial lining showed well marked proliferative changes side by side with desquamation/

desquamation. Fibroblastic growth had also taken place around the ductules and the portal venules so that strands of collagen could be made out extending along the portal tracts around the lobules in the necrotic zones. Side by side with these changes, regenerative activity was also shown by the bile duct epithelium. At first there appeared round the ducts linear strands of oval cells with large ovoid pale-staining nuclei; these were at first arranged in solid masses, but gradually assumed the appearance of tubules with elongated oval cells arranged round a central lumen, like bile canaliculi.

The Effect of Repeated Small Doses.

After 12-45 injections of 7-10 mg. doses thrice weekly amounting to 140-400 mg. of manganese chloride per kilo-weight the changes in the spleen were proli-:ferative and regenerative rather than necrotic. The lesions in the malpighian follicles were variable. Occasionally lymphorrhexis was met with in some, while others showed mitotic division. Periarterial fibrosis and follicular atrophy was sometimes met with, but many follicles showed an increase in size of the marginal zone from a multiplication of the cells at this zone. These dividing cells had pale oval nuclei and resembled germ centre cells of the follicle. Regeneration seemed to commence in two zones, one at the centre of the/

the follicle as in man and the other at the periphery where the change appeared more marked. Erythrophago-:cytic reactions were less marked both in the pulp and the sinuses. Sinus congestion and engorgement of the pulp veins in the chronic cases indicated that the cirrhotic changes in the liver had caused a certain degree of portal stasis. Frequently the sinuses appeared widened and filled with mononuclear and poly-:morphonuclear leucocytes indicating that inflammatory changes were also present. Often these cells could be made out in clusters in the pulp. The nuclei of the syncytium of the pulp appeared more prominent than normal, but on the whole proliferative changes were more marked in the follicles than in the pulp. In five rabbits (Nos.29, 16, 19, 17 and 27) in which the injections were discontinued for a period of six weeks to study the regenerative effects, it was found that the proliferative changes in the follicles reached an extraordinary degree.

In the liver the monolobular fibrosis was still at an early stage with thin strands of inflammatory cells separating the lobules. Here and there con-:densed strands of collagen could be made out around proliferating bile ducts, but necrotic changes were slight. The liver lesions were slighter as compared with the effect of repeated larger doses.

In the kidney congestion of the glomerular tuft and cloudy swelling of the tubules were common.

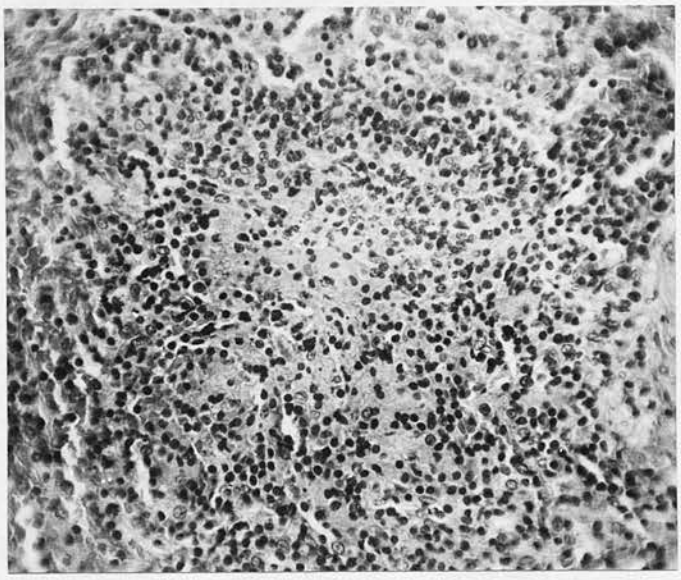


Fig. 1. Rabbit spleen (No.512). 12 subcutaneous injections of 20-30 mg. of manganese chloride; central necrosis of malpighian follicle. (x 300) H and E.

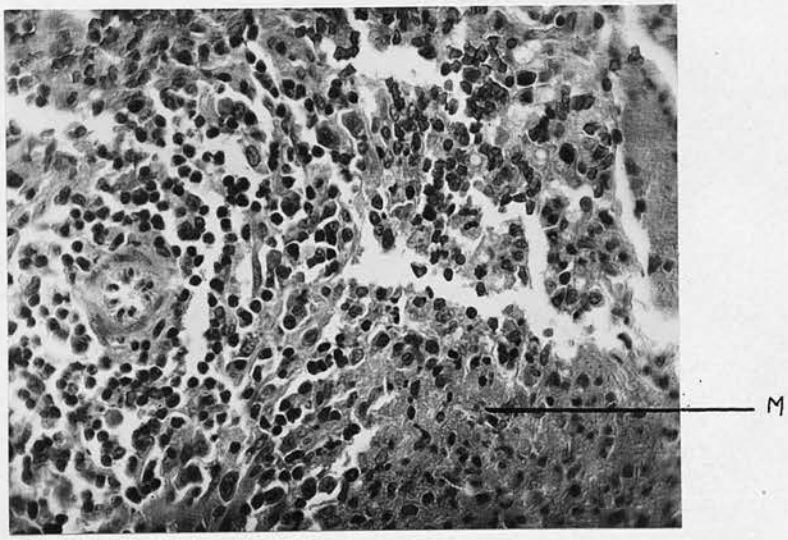


Fig. 2. Rabbit spleen (No.512) after 12 injections of 20-30 mg. of manganese chloride; necrosis at the marginal zone (M). (x 360). H + E

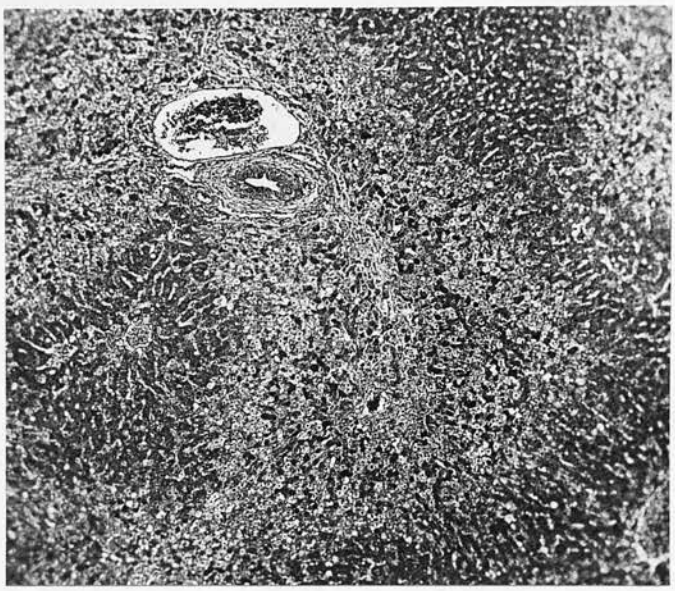


Fig. 3. Rabbit liver (No.512) after 12 injections of 20-30 mg. doses of manganese chloride; necrosis of the peripheral zone of the lobule with early increase of reticulum fibres round the bile duct (x 55). Foot-Wilder.

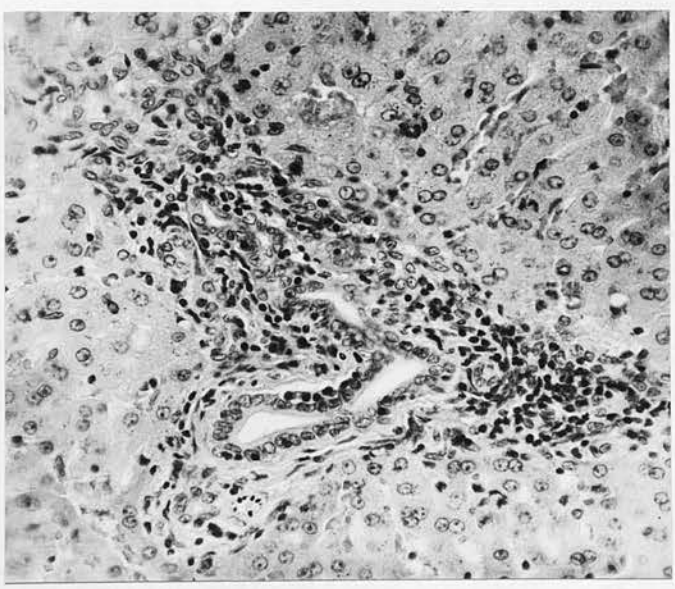


Fig.4. Rabbit liver (No.24) after 16 injections of 10 mg. of manganese chloride; early periductal fibrosis and infiltration with lymphocytes and histiocytes. (x 300). H and E.

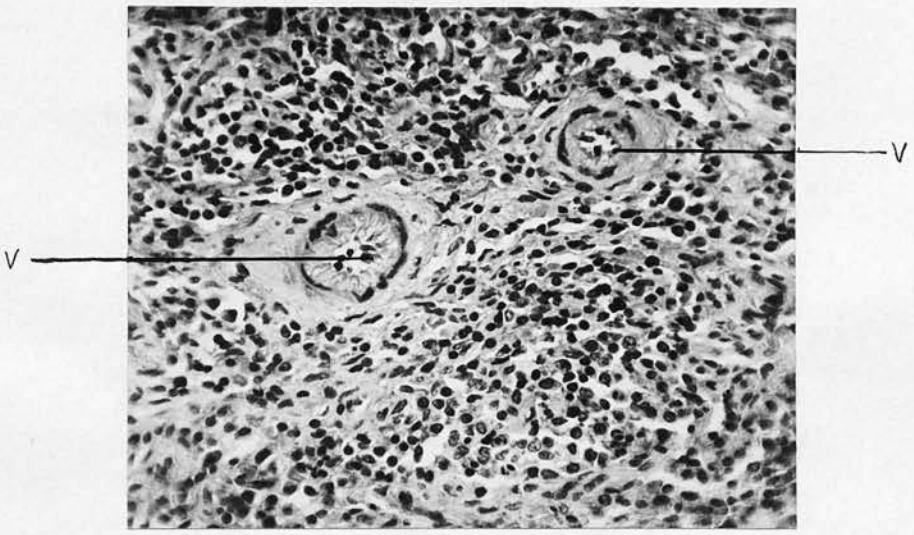


Fig. 5. Rabbit spleen (No.18) after 21 injections of 20 mg. of manganese chloride; periarterial condensa-:tion of reticulum and necrosis of arterial wall; the vascular endothelium (V) is pyknotic. (x 360). H and E.

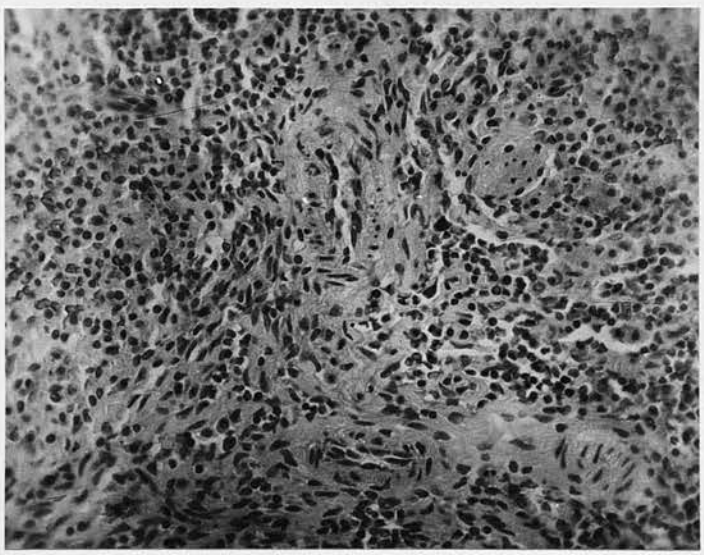


Fig. 6. Rabbit spleen (No.28) after 27 injections of 20 mg. of manganese chloride; early periarterial fibrosis and condensation of the periarterial reticulum; there is marked atrophy of the lymphoid tissue of the malpighian follicle. (x 300). H and E.

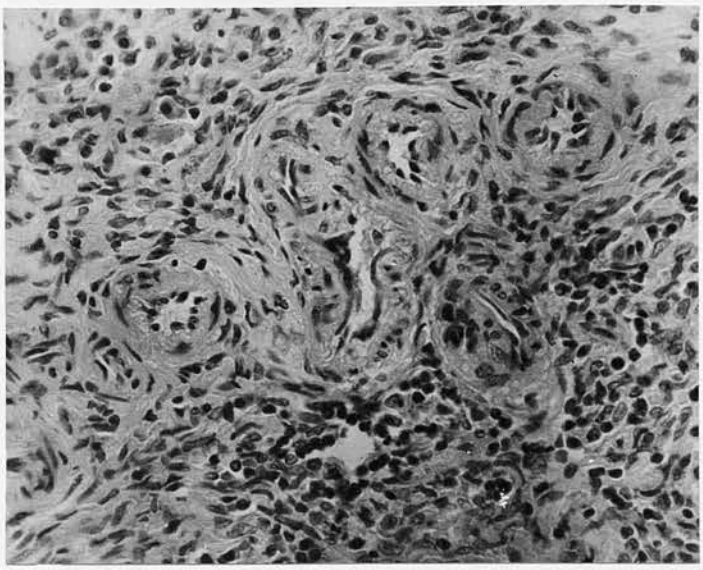


Fig. 7. Rabbit spleen (No.707b) after 12 biweekly doses of 30 mg. of manganese chloride; spread of fibrous tissue from the arterial wall to the follicle; the whole follicle is fibrosed. (x 360). H and E.

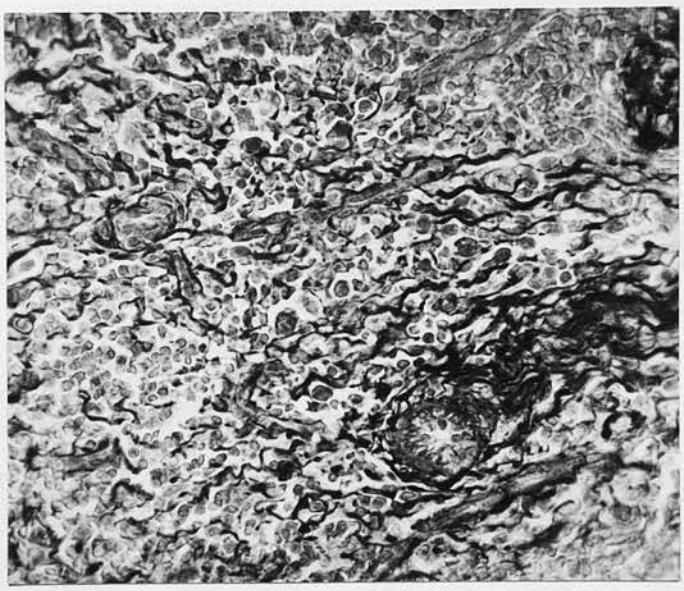


Fig. 8. Rabbit spleen (No.707b) after 12 doses of 30 mg. of manganese chloride; collagenisation of the reticulum; the collagen fibres appear black and thick owing to the deep blue staining. (x 350). Azan.

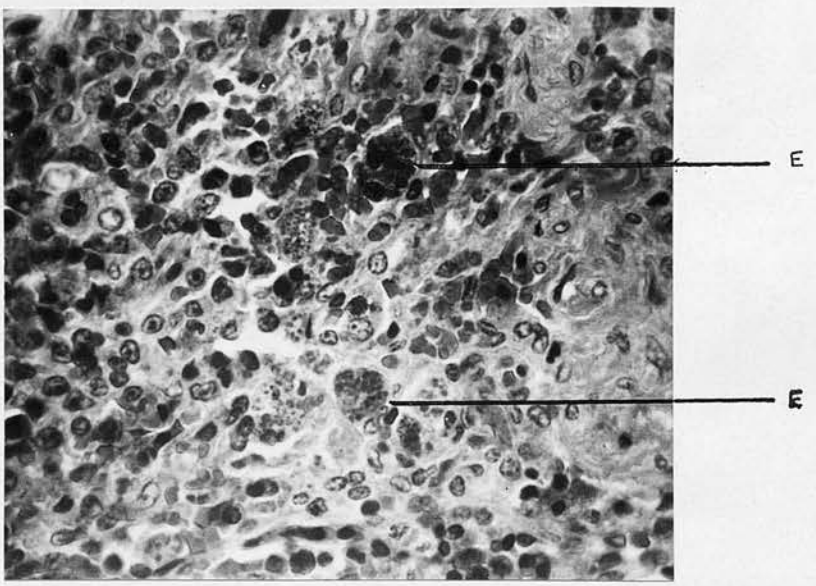
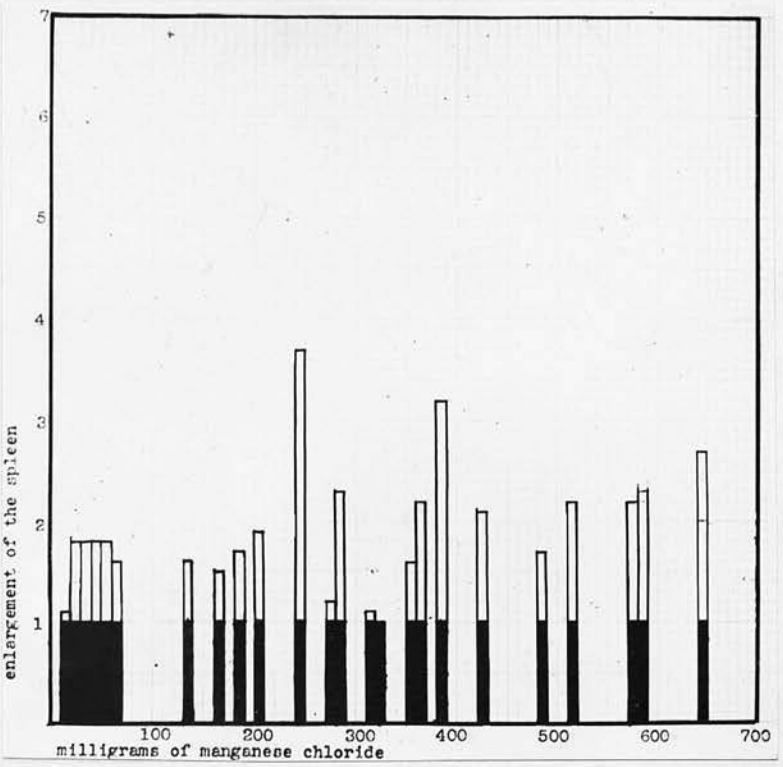


Fig. 9. Rabbit spleen (No.511) after 31 injections of 15-20 mg. of manganese chloride; erythrophagocytic reaction; note the large mono-nuclear cells loaded with disintegrating red cells (erythrophagocytes E). (x 550). H and E.

Fig. 10. Rabbit spleen (No.29) after 45 injections of 7-10 mg. doses of manganese chloride, one month after stopping the injections; extreme hyperplastic regen-:eration of the malpighian follicle. (x 120). H and E.



Histogram 1, showing the degree of splenic enlargement as judged by the spleen weights, in manganese toxaemia in rabbits; the black columns represent the estimated normal weights.

Summary of the Effects of Manganese Toxaemia.

1. In acute manganese toxaemia in rabbits there is marked toxic necrosis of the spleen corresponding to the liver necrosis. This necrotic reaction may be diffuse in the pulp or more localised to the peri-:vascular region.

2. In chronic manganese toxaemia there is evidence of repeated periarterial necrosis followed by fibrillary overgrowth and in the later stages by a spread of fibro-:blastic tissue from the vessels so that areas of peri-arterial fibrosis are formed. These changes in the spleen correspond to the early stages of peripheral necrosis and periductal inflammation in the liver.

3. A splenomegaly of moderate degree is an accom-:paniment of this early pre-cirrhotic toxaemia.

4. With very small doses of manganese and during the stage of recovery from the manganese toxaemia there is marked proliferative enlargement of the spleen. During this stage well marked hyperplastic reactions are found in the malpighian follicles suggestive of the condition called "lymphoid reticulosis".

5. During the toxaemic process there is a marked exaggeration of the erythrophagocytic activity of the spleen with the formation of numerous haematophages which actively ingest red cells. A condition suggestive of a splenic anaemia is thus brought about.

II. CARBON TETRACHLORIDE TOXAEMIA.

Review of Literature.

The use of carbon tetrachloride therapeutically as an anthelmentic and in industry for various purposes has resulted in the occurrence of cases of fatal poisoning and sometimes of cirrhosis of the liver in man. While the pathological changes in the liver and kidneys have been dealt with in detail little or no evidence of splenic damage is recorded. Thus Clayton Lane (1932) in his work on hookworm infection, while he discusses the lesions caused by the drug in fatal cases, mentions that the spleen is not involved. In autopsy cases of acute poisoning, and after administration of toxic doses to criminals prior to execution (Docherty and Burgess, 1922; Lambert, 1923; Docherty and Nicolls, 1923; Straub, 1925; Minot, 1927; Lamson, Minot and Robbins, 1928; Blackie, 1931; McMohan and Weiss, 1929; Lattes, 1934; van Scheurlin and Witzky, 1935) attention has been mostly directed to changes in the liver and kidneys and occasionally the adrenals. Phelps and Hu (1924) mention splenic changes in one case. There were foci of haemorrhage in the spleen, and in addition there was a diffuse infiltration of the pulp with plasma cells and endothelial leucocytes showing phagocytosis of brownish pigment. Congestion of/

of the spleen and haemorrhages from the stomach and petichiae in serous membranes besides liver necrosis are reported by Kehrer and Oudendal (1926) following the use of the drug as an anthelmentic. Well defined chronic splenomegaly in man is also recorded by Poindexter and Green (1934) in a case of cirrhosis following chronic poisoning with carbon tetrachloride. Histologically the spleen showed atrophy of the malpighian bodies, dilatation of the sinuses and an increase in the reticulum of the pulp.

In experimental work, the effects of carbon tetrachloride have been studied by injection, after oral administration, and following inhalation of the vapour.

Lesions in the dog have been extensively studied. While earlier experiments (Hall, 1921; 1922) were carried out to determine the degree of safety of therapeutic doses and the effect as an anthelmentic, study of the toxic action was carried out by Mayer and Pessôa (1922), Smillie and Pessôa (1923), Schultz and Marx (1924), Gardner and his coworkers (1925), Mann, Fishback, Gay and Green (1931), Bollmann and Mann (1931) and many others. These studies have brought to light the frequency of necrosis of the liver. Lesions in the kidneys, varying from engorgement to swelling of epithelium of Bowman's capsule and fatty degeneration of the tubules have been recorded by Chandler and

Chopra/

Chopra (1926-27) in addition to the hepatic lesions.

In the rabbit, Lehmann (1911) noticed fatty degeneration in the liver and kidneys after inhalation of the vapour while Davis (1934) described congestion of mucous membranes and central necrosis of the liver in exposed animals. Laudé and Davilleé (1934) pro-:duced changes like acute yellow atrophy after oral administration. While in the kidneys various degrees of fatty degeneration and even necrosis are recorded little mention of splenic changes is found. Okano (1929) however has recorded congestion in the spleen. In biochemical studies, Takahashi (1929) found that disturbance of the nitrogenous metabolism kept pace with the liver necrosis. Of other lesions, Biancalini (1934) has recorded degenerative chromatolysis and vacuolation of the ganglion cells in the nervous system.

In the guineapig, toxic effects have been recorded by Phelps and Hu (1924). They have described the development and the healing of the liver necrosis, the renal lesions and the occasional changes in the adrenals, but no splenic changes. Smyth and Smyth (1936) found that of all laboratory animals guineapigs are the most susceptible and easily killed by inhalation.

In the rat, Lacquet (1932) and Cameron and Karunaratne (1936) have produced all the changes of liver necrosis and cirrhosis. In mice, rabbits and guineapigs/

guineapigs Browning (1937) has found changes similar to acute yellow atrophy of the liver with marked lesions in the kidneys.

Of all these experimental studies mention of any significant splenic changes is found only in the work of Smyth and Smyth (1936). They noticed an extensive destruction of the red blood cells in the spleen of experimental animals.

Material and Methods.

Thirty-two young albino rats together with eight controls, and eighteen guineapigs with four controls were used in the course of these experiments with carbon tetrachloride. The animals were kept under the same conditions and were fed on the same diet. Great care was taken to avoid any cage infection by cleaning and disinfection of the cages twice a week, as it was thought that any infective process might vitiate the results and interfere with the condition of the spleen.

For rats pure carbon tetrachloride was given undiluted by means of a tuberculin syringe in doses varying from 0.08 to 0.15 c.c. every third day, doses slightly less than those of Cameron and Karunaratne (1936). It was noticed that in the beginning doses larger than 0.1 c.c. caused local necrosis at the site of injection, but if the needle was introduced deep into/

into the subcutaneous tissue and the fluid spread out by massage, larger doses could be injected, without much local damage. The injections were given into the skin of the abdominal wall nearer the middle line where the tissues are very lax and the skin is rather thin. Injections into the tail were invariably followed by necrosis. For guineapigs doses ranging from 0.15 to 0.25 c.c. were used but were so toxic as to cause death in 2 to 6 days. So the effect of repeated injections could not be determined.

For purposes of histological examination of the lesions the animals were killed by dislocation of the neck and not by ether anaesthesia as it was found that respiratory failure interfered with the vascular state of the spleen. The whole spleen, the splenic vessels, portions of the liver, the kidneys, the heart muscle and the adrenals were removed for histological examination and dealt with as described in experiments with manganese.

The Normal Spleen of the Rat.

While the spleen of the albino rat is capable of variations in size and weight both physiologically from circulatory factors and as a result of cage infections, it is possible to arrive at an estimate of the average size and weight for experimental purposes, if care is taken to select healthy animals free from anaemia and

if cage infections are prevented. It is therefore not surprising that the table showing the spleen weights of the control animals shows more or less parallel figures to what Hatai (1913) claimed to be the normal variation of spleen weight to body weight. Jackson (1915) found a similar figure of 0.27% of the body weight and this has been used in estimating normal weights in protocol I.

Histologically, the rat's spleen differs from the human in that the malpighian follicles are relatively larger and fewer in number and are arranged in a cen-:tral longitudinal row in the medulla of the organ. The follicle itself is divided into three zones, a central which contains aggregations of large and small lymphocytes, an intermediate of reticulo-endothelium in which runs a thick-walled annular capillary mesh, and a marginal zone of pale oval cells in which a few lymphocytes are present. When the follicles are active a central germ centre appears, and the marginal zone is widened. The eccentric arteriole is a thick-walled vessel which is generally more prominent than in man. The lymphoid tissue is not however confined to the malpighian follicles, but is spread out as irregular clusters of cells in the pulp and around the smaller arterioles and the trabeculae. In old rats plasma cells are met with, though it is uncertain how far/

far they can be regarded as physiological. Megakaryo-:cytes are more frequent than in the human spleen. The capsulo-trabecular system is thin and the venous tissue appears comparatively reduced. The trabecular veins and pulp veins are frequently lined by lymphocytes in the subendothelial layer. Occasionally a few phagocytic mononuclear cells containing ingested red blood cells are met with and sometimes clusters of haemosiderin granules are found in the trabeculae. The granular leucocytes are found only in proportion to what are found in the blood. A few myelocytes are also commonly met with. The degree of congestion as judged by the distension of the sinuses is a variable factor that seems to depend on the state of the muscula-:ture of the spleen as regards contraction or relaxa-:tion. If death has been induced as a result of ether anaesthesia the organ is flaccid and the venous system generally distended.

The Normal Guineapig's Spleen.

The spleen of the guineapig is an obovate leaf-like organ that is slightly thicker and more fleshy than the rabbit's spleen. It has a light pink colour that is rather different from the purplish colour of the spleen of other animals. The capsule is trans-:parent and shiney and often wrinkled. Krumbhaar (1926)/

(1926) gives the spleen weight as 0.13% of the body weight. Histologically the capsulo-trabecular system is remarkable for the amount of muscle fibres and elastic tissue that is much more abundant than in the rabbit's or the rat's spleen. The malpighian follicles are also peculiar in that the lymphoid tissue is spread out as oval sheaths rather than as rounded swellings along the terminal arterioles. The reticulo-endothelium of the pulp is much more developed than in the rat or rabbit and consequently the cords of Billroth are crowded with nuclei while the sinuses are wider apart and the sinus tissue considerably reduced. Cells derived from the reticulo-endothelium are occasionally met with free in the malpighian follicles. Megakaryocytes are also frequent and haematophages are also sometimes found.

Results.

General Effects.

The first effect that was noticed was cessation of breeding. Some animals showed progressive loss of weight, the skin became dry and the hair staring. These changes were more marked in the later stages of the experiments after injections had been continued for a month. Some animals remained comparatively healthy and in one, extreme adiposity was noticed. This variation in the individual resistance was a puzzling/

puzzling factor and was noticeable throughout the experiments. The animals were fed on the same diet and the factor of any calcium excess as suggested by Minot (1927) and by Smyth and Smyth (1936) as favouring resistance to the action of the drug could not be im-:plicated from the diet. With repeated injections after about 12-16 weeks most of the animals developed ascites, the urine was found to be high coloured. Jaundice was exceptional. The effect of the drug on the liver as judged by the development of ascites seemed to vary in animals getting the same dose. With larger doses the animals passed into a condition of coma ending in death.

The Effect of a Single Large Dose of 0.5 c.c.

The spleen showed a definite necrotic reaction of the cytoplasmic syncytium; the appearance was more or less similar to that found with large doses of mangan-:ese except that the malpighian follicles were not specially picked out. The swelling of the syncytium and the filling in of the pulp mesh and necrotic changes in the nuclei are all brought out in Fig.11. Degenerative swelling of the pulp has been little understood in the spleen and there has been a tendency to look upon this as an autolytic change (Perla and Marmorsten, 1935), but here the grading of the reaction with different degrees of toxic damage indicates that the/

the mesenchymal tissue of the spleen is just as liable to undergo toxic necrosis as paranchymatous cells.

The liver, 24 hours after the injection showed early central necrosis as suggested by pyknosis and karyorrhexis of the nuclei and diffuse eosinophilic staining of the cytoplasm; many of the cells were swollen and showed varying grades of hydropic degenera-:tion as described by Cameron and Karunaratne (1936). The cells at the periphery and the middle zone were more or less normal and with Leishman's stain their granular cytoplasm could be demonstrated. Their nuclei appeared more prominent and active. Congestion was variable. Haemorrhages into the necrotic zone were sometimes present.

The kidneys showed slight cloudy swelling of the epithelium of the convoluted tubules.

The Effect of a Single Dose of 0.2 c.c.

In 24 hours the spleen showed a variable, but diffuse congestion; there was slight lymphorrhexis in the malpighian follicles; the syncytium of the pulp was somewhat swollen and showed slight condensation and opacity of the cytoplasm; occasionally the nuclei appeared more opaque and pyknotic; as a result of the swelling of the syncytium the pulp mesh was very much reduced, the gaps appeared filled in. In the liver, areas of central necrosis were distinct in some animals, but/

but not marked in others; the effect seemed to vary a good deal in intensity in different animals of the same species. The kidneys showed active congestion of the glomerular tuft, swelling of the epithelium of Bowman's capsule and fatty degeneration of the epithelium of the convuluted tubules.

The Effect of 3 Doses of 0.2 c.c. every Two Days.

When the dose was repeated three times the effects were much more distinct. The spleen showed as before, a variable congestion, that suggested an active hyper-:aemia; the malpighian follicles showed a distinct hyperplasia of the marginal zone which spread out as an annular band about 4 to 5 layers in thickness; the cells that formed this zone were pale staining cells with oval nuclei with a thin chromatin mesh work; the appearance was more like that of endothelioid cells derived from the reticulum rather than lymphoblasts; in the centre of the follicle "germ centres" appeared and the process of formation of lymphocytes was sugges-:ted by the appearance of numerous round cells with deep staining opaque nuclei; these cells were mostly confined to the central zone; in places cells with basophilic cytoplasm and larger nuclei suggested that plasma cells were also formed. Necrotic follicle changes were not marked. In the pulp, small circum-:scribed areas of mononuclear cells and plasma cells

which/

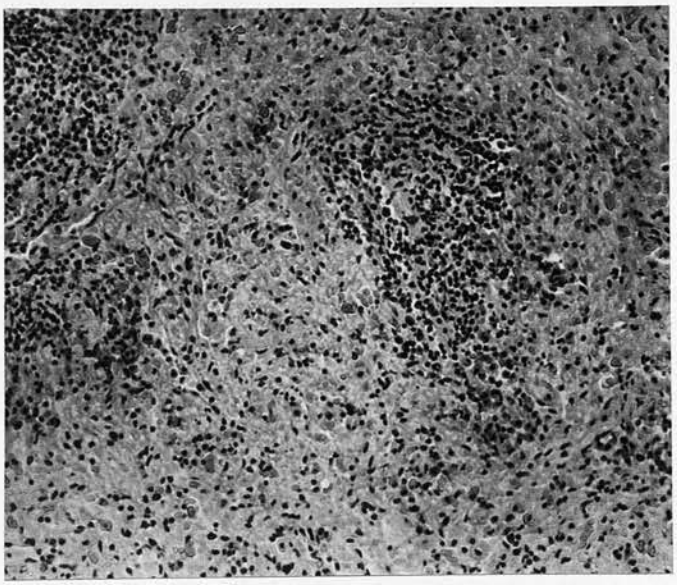


Fig. 11. Rat spleen after 8 injections of 0.1-0.2 c.c. of carbon tetrachloride; extensive necrosis of the reticular syncytium of the pulp and of the reticulum of the malpighian follicles. (x 175). H and E.

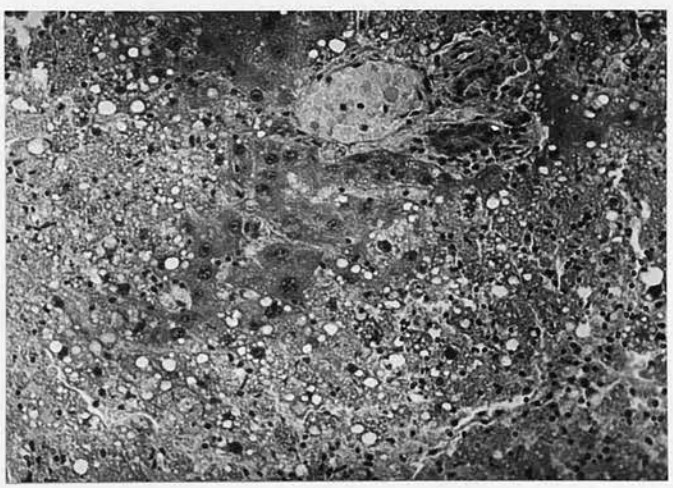


Fig. 12. Rat liver after 8 injections of 0.1-0.2 c.c. of carbon tetrachloride; extensive necrosis and hydropic degeneration of the central and middle zones with only a few comparatively healthy liver cells around the portal tracts. (x 175). H and E.

Fig. 13. Rat liver after 30 injections of 0.1 c.c. of carbon tetrachloride showing the multilobular cirrhosis. (x 60). Azan.

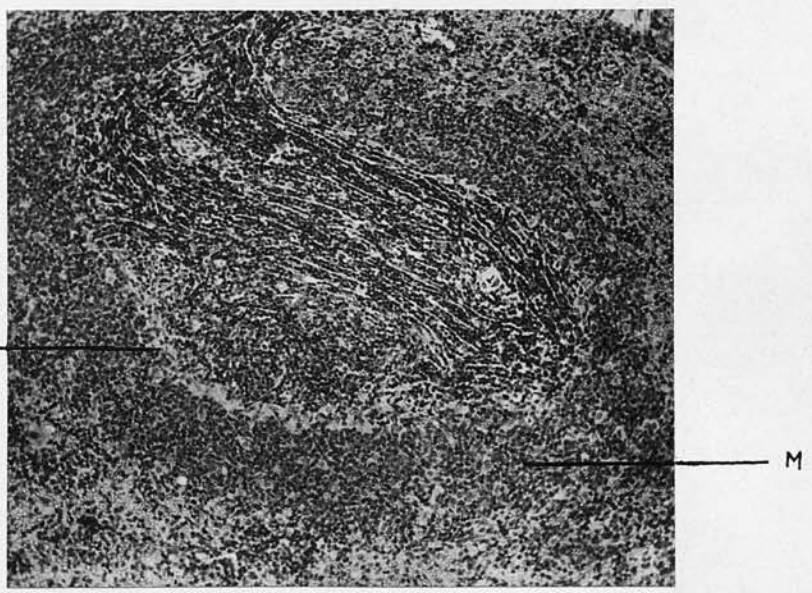


Fig. 14. Rat spleen after 34 injections of 0.1 c.c. of carbon tetrachloride; marked hyperplasia of the malpighian follicle with widening of the marginal zone (M) from proliferation of reticulum cells. (C) shows the capillary mesh. (x 120). H and E.

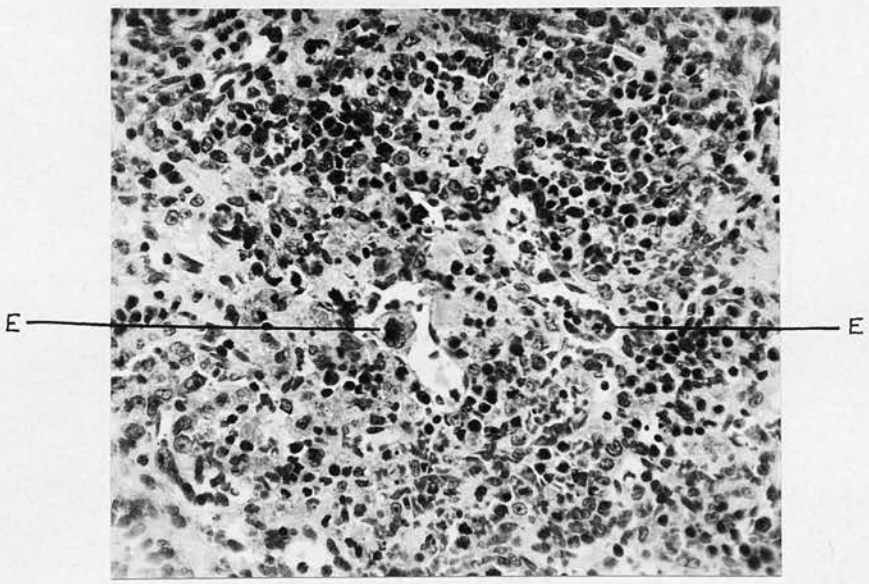


Fig. 15. Rat spleen after 48 injections of 0.1-0.15 c.c. of carbon tetrachloride showing the formation of erythrophagocytes (E) and inflammatory cells in the pulp. (x 300). H and E.

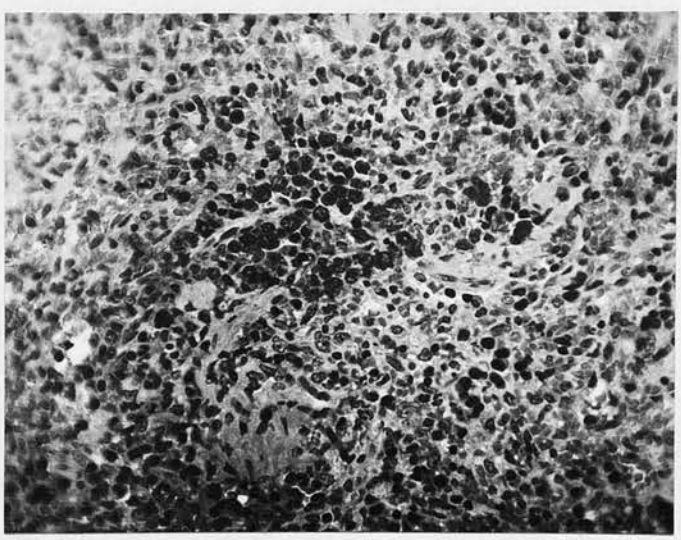


Fig.16. Rat spleen after 48 injections of 0.1-0.15 c.c. of carbon tetrachloride showing the focal inflamma-:tory clusters in the pulp; some of the cells are eosinophiles. (x 300).

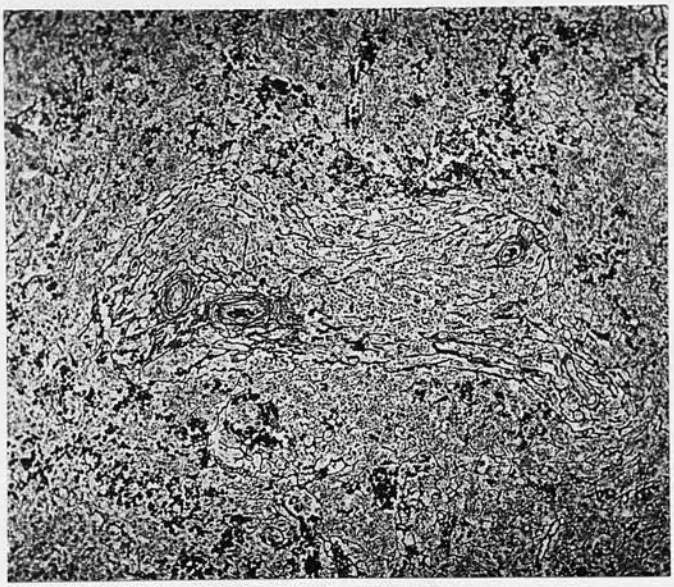


Fig.17. Rat spleen after 45 injections of 0.1 c.c. of carbon tetrachloride showing periarterial fibrillary increase in a malpighian follicle. (x 120). Foot-Wilder stain.

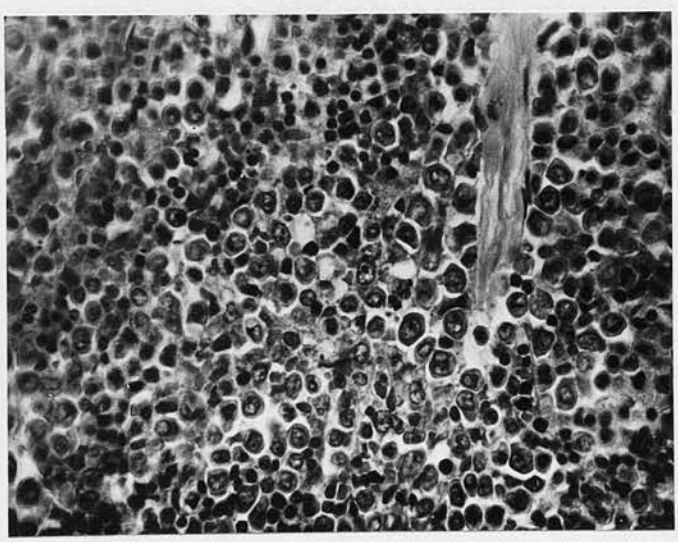


Fig.18. Rat spleen after 45 injections of 0.1 c.c. of carbon tetrachloride showing extreme cellular hyper-:plasia and the formation of mononuclear cells. (x 390). H and E.

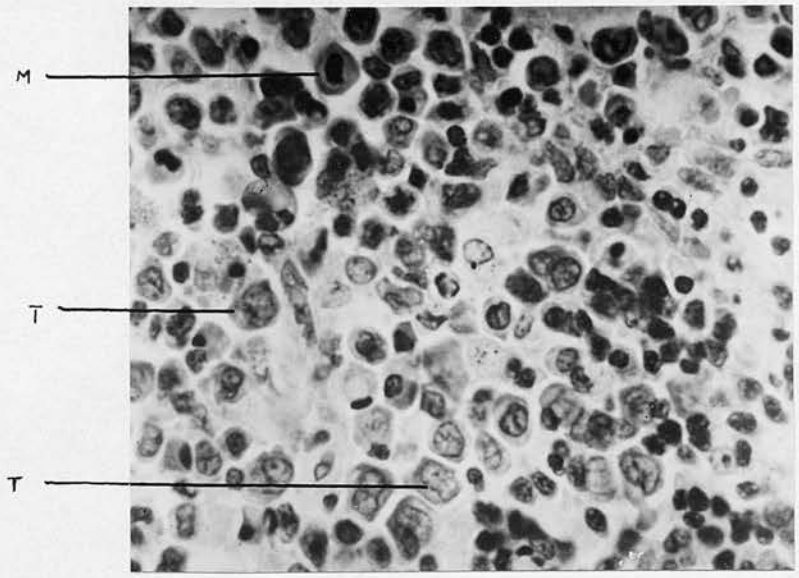


Fig.19. Rat spleen after 28 injections of carbon tetrachloride showing an extreme hyperplastic response and the formation of numerous free mononuclear cells, some with an indented nucleus (T), and some showing mitosis (M). This response is diffuse throughout the whole spleen pulp. (x 700). Leishman.

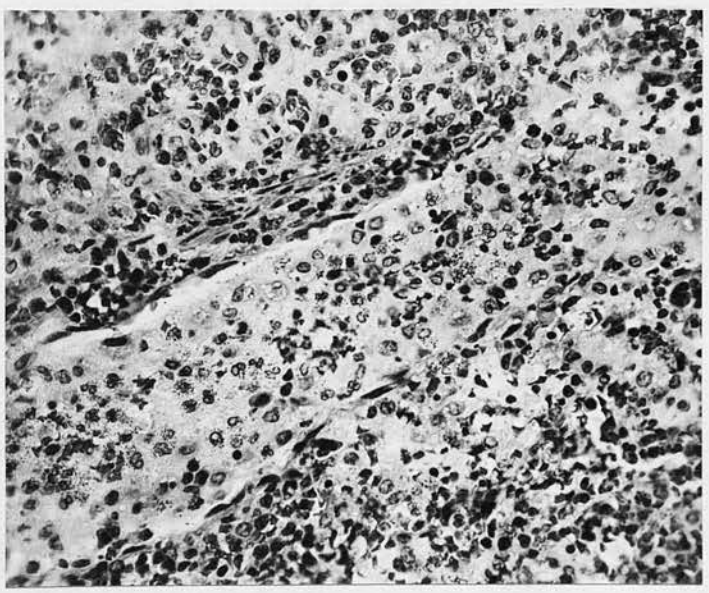


Fig.20. Guineapig spleen after two injections of 0.15 c.c. of carbon tetrachloride showing the distension of a pulp vein with mononuclear and neutrophile leucocytes; note the inflammatory cells in the pulp. (x 310). H and E.

which are normally found in the rat spleen, seemed to be undergoing active proliferation; these clusters became more focal in distribution and grouped around the smaller arterial capillaries and around the tra-:beculae; each cell cluster was formed by a basic group of proliferating histiocytes; in between these cells were the basophilic cells resembling large plasma cells; numerous large and small lymphocytes were intermingled. Necrotic changes in the syncytium could be made out side by side with proliferative regeneration. The capsulo-trabecular system seemed rather thin and stretched and occasionally trabecular softening and necrosis could be made out; vascular changes were not definite.

In the liver the necrotic areas were more irregu-:lar and seemed to radiate in irregular extensions from the centre of the lobule; often they ran on in bands from one central zone to another in a neigh-:bouring lobule as in venous congestion, probably more in relation to the sublobular veins. A few leucocytes could be seen to have wandered into the necrotic zone. The Küpffer cells in the necrotic area appeared more prominent, but this was from degenerative swelling of the cytoplasm and a condensation of the nucleus rather than from any active proliferative change.

The/

The Effect of Repeated Small Doses.

With 10-30 injections of 0.1-0.15 c.c. doses of carbon tetrachloride amounting to 1.8 to 3.3.c.c. in two to ten weeks the reactions in the spleen were more or less similar. Reticulo-endothelial hyperplasia was shown by marked reactive changes in the marginal zone of the malpighian follicles, focal collections of basophilic cells were more marked in the pulp, and in places there was a suggestion of early fibrosis in the pulp. Necrotic reactions were not marked. Erythro-:phagocytosis was more active than in the normal rat spleen; megakaryocytes were commonly met with, but as they are frequently met with in the rat's spleen little significance could be attached to their presence.

In the liver, a condensation of the reticulum in the necrotic areas was followed by a gradual spread of fibrous tissue into the collapsed areas indicating the commencement of active fibrosis. The newly formed fibrous tissue was fibroblastic and collagen fibres were very thin and scanty. An irregular pattern of multilobular fibrosis was thus evolved by irregular spread from the portal tracts into the collapsed areas; the bile ducts showed slight proliferative activity. The remarkable feature however, was the spread of regenerative activity that seemed to go on even with the earlier stage of the necrotic process; around/

around the necrotic areas many of the liver cells showed hyperchromasia, and giant nuclei often with condensed chromatin. Their cytoplasm was deeply basophilic. These cells were often found at the periphery of the lobule, but often also in the middle zone and around the necrotic areas.

With 30-52 injections of 0.08 to 0.15 c.c. of carbon tetrachloride amounting to 3.4 to 5.9 c.c. in 10 to 25 weeks the injections being given thrice and twice weekly, the changes in the spleen were compli-:cated by venous congestion caused by portal obstruc-:tion. This was shown by the engorgement of the trabecular veins, the pulp veins and the sinuses. The sinus congestion had also caused diffuse percola-:tion of blood in the centre of the lobule. A pro-:liferative growth of sinus tissue was not met with. Under the capsule, the sinuses were dilated and their walls appeared thin and more rigid than normal owing to a spread of fibrous strands from the capsule, but this was never marked. The malpighian follicles showed well marked hyperplastic reactions as in the earlier stages. The cellular foci in the pulp were more diffuse and it seemed as if the reticulo-endo-:thelial reaction had formed a free macrophage tissue in which numerous basophilic cells were present.

In/

In between the pale staining pulp nuclei, lymphocytes and basophilic cells, and clusters of eosinophilic and neutrophilic leucocytes were present. Many of the free cells had abundant cytoplasm which was faintly basophilic as in the normal mononuclear macrophage cell. Erythrophagocytosis was more marked than normal.

In two rats Nos.III.1 and IV.3 after 28 and 45 injections the proliferative reaction and mononuclear differentiation was extreme. The whole pulp was filled with numerous large round cells which were not myelocytes but non-granular cells with large oval nuclei which were often kidney shaped (see Figs.18 and 19). Side by side with the extreme proliferative activity, a gradual increase in the fibrillary reticu-;lum could be made out with the Foot-Wilder stain (Fig.17). The hyperplastic reaction was however mostly in the pulp and had not affected the malpighian follicles which appeared compressed by the alteration in the pulp.

The liver showed macroscopically well marked cirrhosis; the appearance however varied from a fine irregular granularity to the formation of coarse nodules of variable size as in the hob-nailed liver (Figs.21 and 22). In between the nodules condensed pinkish bands could be seen on the surface and on section;

some/

some of the nodules on the surface were soft, white and almost cystic. On section the tissue was more difficult to cut and the cut surface showed an irregu-:lar lobulation as in the human liver in Laennec's cirrhosis. Occasionally capsular thickening was well marked. Histologically, there was a well marked multilobular cirrhosis as shown by the spread of strands of fibrous tissue breaking up the lobules. These were well brought out by Heidenhain's azan stain. In the fibrous strands, foci of lymphocytes and histiocytes were frequent; bile duct proliferation was quite common, but there was no definite periductal inflammation as in manganese cirrhosis. The liver cells inside the irregular lobules showed fatty de-:generation. Occasionally they appeared fused to-:gether so that cell boundaries were indistinct. In many places, the sinusoidal system had disappeared, but occasionally compressed and distorted remnants of Kupffer cells could be seen in between the hepatic cells. In animals that were killed soon after the injections were stopped, the necrotic reaction could be seen around the thin collagen bands. In all cases regenerative changes were very marked. Where injec-:tions had been discontinued for more than a week the liver cells showed glycogen storage. In the two cases that showed hyperplasia in the spleen the liver showed

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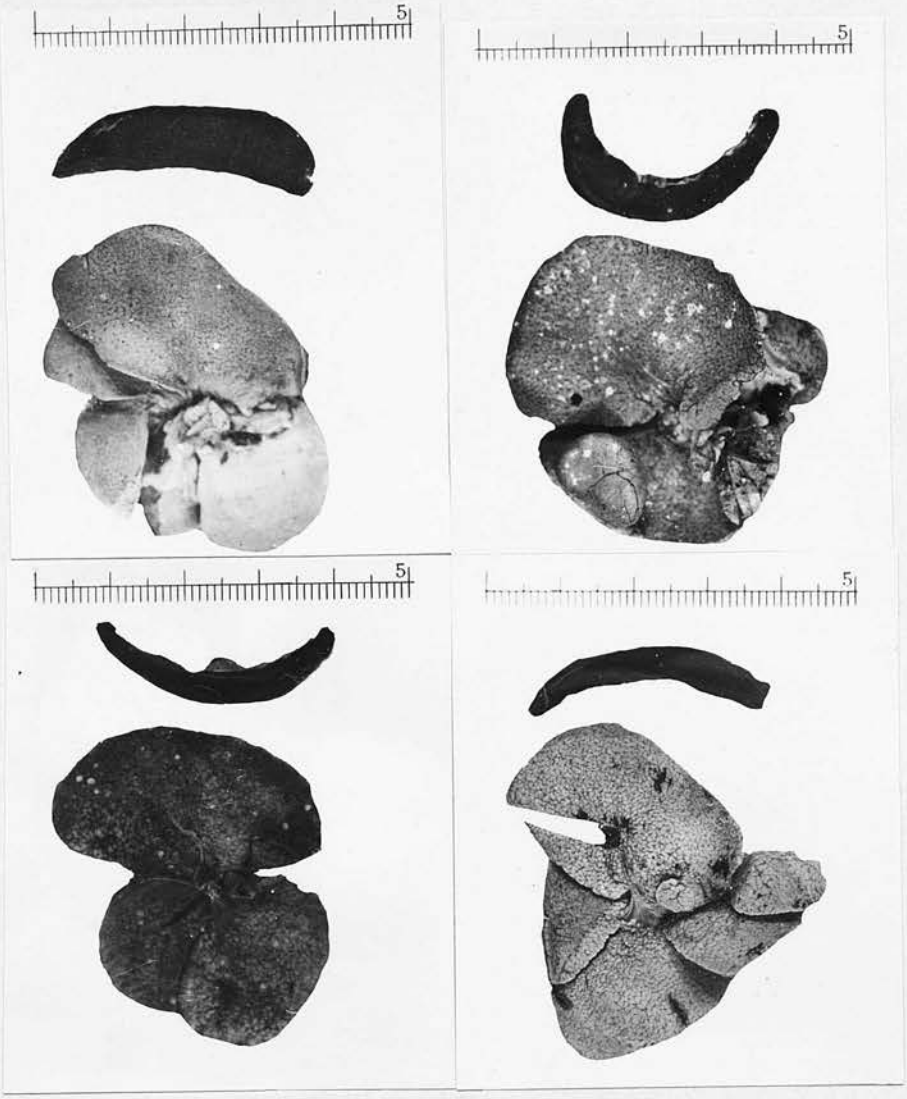


Fig.21, showing the early stages of carbon tetrachloride cirrhosis. Note the exaggeration of the lobular pattern and the development of a fine granularity with the formation of coarser hypertrophied nodules projecting from the surface. The splenomegaly is most marked in the precirrhotic stage.

Fig.22, showing the development of coarse cirrhosis after thirty to 50 injections of carbon tetrachloride. The splenomegaly is quite well marked.

Protocol of Animal Experiments. II.

Carbon Tetra-chloride Toxaemia in Rats.

Animal No.	No. of injec-:tions	Total dosage in c.c.	Body Wt. in gm.	Size of spleen in c.m.	Spleen Wt. in gm.	Estima-:ted normal wt.	Wt.ratio (enlarge-:ment)	Remarks
VIIL3.	1	0.5	160	3.3-0.7-0.25	0.68	0.43	1.4	24 hrs
VIIL4.	1	0.5	170	2.7-0.7-0.25	0.59	0.46	1.3	24 hrs
VIIL1.	1	0.5	140	4.1-1.0-0.4	1.15	0.38	3.0	48 hrs
VIII2.	1	0.5	230	4.2-1.0-0.4	1.05	0.62	1.7	48 hrs
VIII.	1	0.2	160	3.1-0.5-0.3	0.69	0.43	1.6	
I.1.	2	0.4	100	2.5-0.6-0.3	0.29	0.27	1.1	
III.4.	3	0.6	190	3.0-0.9-0.5	0.84	0.51	1.6	
IV.1.	3	0.7	220	5.1-1.4-0.7	3.09	0.59	5.3	
VI.4.	7	1.4	200	4.5-1.4-0.7	2.65	0.54	4.9	
II.3.	8	1.5	180	3.5-1.0-0.5	1.19	0.49	2.4	
VI.2.	10	1.8	170	3.5-1.1-0.5	1.18	0.46	2.6	
I.2.	10	1.8	180	3.5-0.9-0.4	0.86	0.49	1.8	
V.1.	10	1.8	100	3.2-1.0-0.3	0.85	0.27	3.1	
I.4.	11	1.9	110	3.5-0.9-0.5	0.98	0.30	3.3	
VII.1.	11	1.9	120	3.7-1.0-0.5	0.73	0.32	2.3	
VI.3.	12	2.0	190	3.5-0.9-0.5	1.15	0.51	2.3	
II.1.	16	2.3	170	3.5-0.8-0.4	0.90	0.46	1.9	
III.1.	28	3.1	150	4.5-1.3-0.3	1.25	0.41	3.0	extreme hyperplasia
V.3.	30	3.3	160	4.0-1.0-0.3	1.33	0.45	3.0	
VI.1.	31	3.4	150	4.0-1.1-0.5	1.45	0.41	3.5	

V.4./

Protocol II (continued)

Animal No.	No. of injections	Total dosage in c.c.	Body Wt. in gm.	Size of spleen in c.m.	Spleen Wt. in gm.	Estimated normal wt.	Wt. ratio (enlargement)	Remarks
V.4.	34	3.7	180	4.2-1.0-0.3	1.15	0.49	2.3	
V.2.	35	3.8	150	3.3-1.2-0.4	0.97	0.41	2.4	
III.2.	35	3.8	130	3.2-1.0-0.35	0.88	0.35	2.5	
II.2.	35	3.8	110	3.1-1.0-0.4	0.94	0.30	3.1	
VII.2.	38	4.1	130	4.3-0.6-0.25	0.81	0.35	2.3	
VII.3.	43	4.7	150	3.7-0.9-0.3	0.80	0.41	2.0	
IV.3.	45	5.0	150	4.8-1.2-0.4	2.45	0.41	6.0	Extreme hyperplasia
VII.4.	48	5.3	180	3.2-0.9-0.35	0.80	0.49	1.6	
I.3.	52	5.9	200	3.8-0.9-0.3	0.81	0.54	1.5	
II.4.	52	5.9	300	4.1-1.2-0.35	1.58	0.81	1.9	Extreme adiposity
III.3.	52	5.9	220	4.1-1.1-0.3	1.0	0.59	1.7	
IV.4.	52	5.9	180	4.1-1.0-0.3	1.18	0.49	2.4	

Control Rats.

Animal No.	Body Weight	Size of spleen	Spleen Weight	Estimated normal weight
N.1	165	3.1-0.6-0.3	0.45	0.44
N.2	195	3.1-0.7-0.3	0.46	0.52
N.3	210	3.2-0.8-0.3	0.64	0.57 (pregnant)
N.4	185	3.1-0.6-0.3	0.43	0.50
N.5	150	3.1-0.7-0.2	0.44	0.40
N.6	185	3.2-0.7-0.3	0.51	0.50
N.7	210	3.2-0.7-0.3	0.54	0.57
N.8	180	3.2-0.7-0.3	0.52	0.49

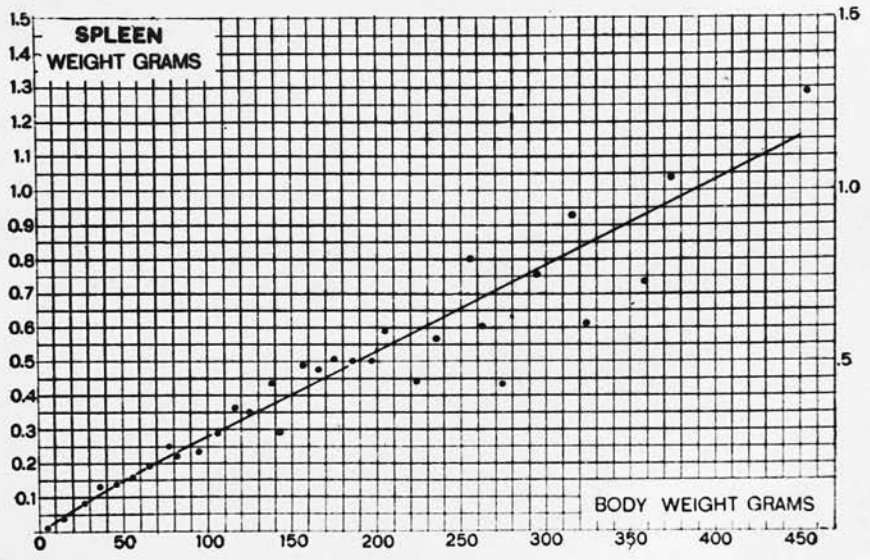
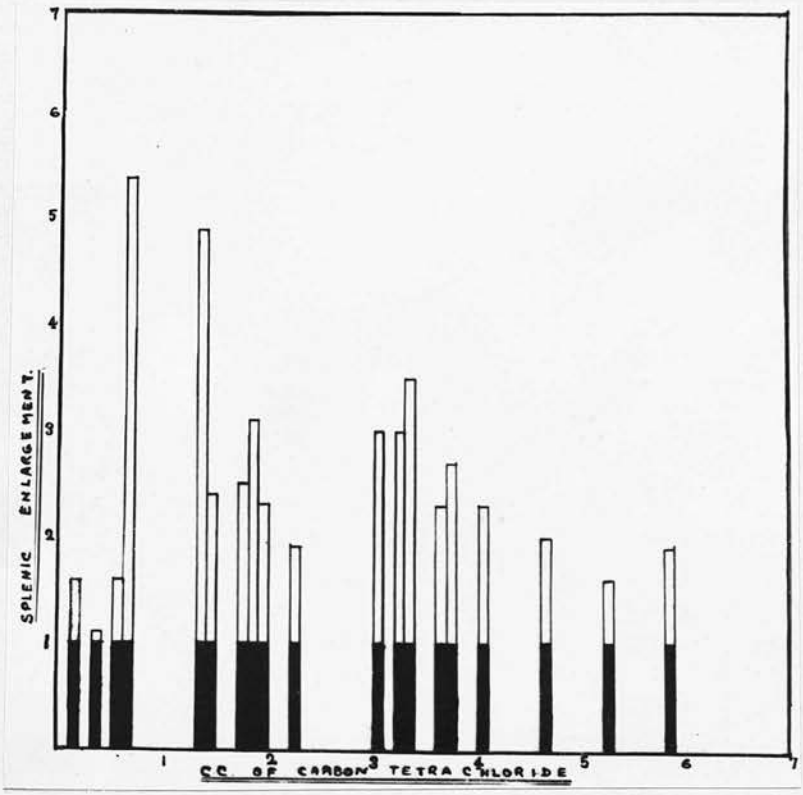


Chart 5 Showing the weight of spleen of the male albino rat according to body weight. The observed weights are represented by 87 male rats.
● Observed weight. —— Calculated weight.

Hatai's curve showing the variation in spleen weight in the normal albino rat.



Histogram II showing the degree of splenic enlarge-:ment as judged by the spleen weight in carbon tetrachloride toxaemia in rats; the black columns represent estimated normal weights. Note the pre-cirrhotic trend of the splenomegaly (one case showing extreme hyperplasia to about six times the normal is not included in this chart).

a commencing cirrhosis.

Carbon Tetrachloride Toxaemia in Guineapigs.

Carbon tetrachloride in doses of 0.15-0.20 was found to be so toxic as to cause death in two days in 8 animals. After two injections of 0.1 c.c. death occurred in two animals in 6 days. The effect of repeated small doses could not therefore be determined even though Van der Scheuren (1932) claims to have produced cirrhosis with doses of 0.25 c.c. twice weekly for four weeks.

The Effects of Carbon Tetrachloride in Guineapigs.

In animals that died 48 nours after the adminis-:tration of 0.1-0.20 c.c. the changes were as follows: in the spleen the type of reaction was slightly differ-:ent from that met with in rats; the rarity of necrotic follicle reactions was however similar. Necrotic reactions in the pulp syncytium were slight and more focal than in rats after large doses. Engorgement of the spleen was not met with. On the other hand, there was a widespread cell accumulation that was constant. Histiocytic differentiation was quite marked in the pulp syncytium and littoral cell proliferation was marked in the sinuses. Many free mononuclear cells thus appeared in the pulp; very often there were focal clusters with numerous polymorphs and lymphocytes, both/

both in the sinuses and around the vessels. Clusters of basophilic cells similar to those met with in the rat's spleen were not commonly met with. Capsulo-trabecular changes were not distinct and pulp fibrosis was not met with. That the cellular reaction was a primary response to the toxic agent was certain as there was no complicating sepsis either at the site of injection or in the other viscera. Besides the reaction was constant in all the animals. In 6 days after injection, the reaction had subsided.

The changes in the liver in 48 hours were more or less similar to those met with in rats and consisted in central islands of necrosis which showed a tendency to spread irregularly from one central zone to another. Hydropic degeneration of the liver cells around the necrotic zone was also quite common. Infiltration of the necrotic zone with polymorphonuclear leucocytes was frequently met with indicating a marked absorptive activity. In 6 days signs of regeneration were well marked and the absorption of the necrotic foci was almost complete.

In the kidney the glomeruli were distended with blood indicating an active hyperaemia. The tubular epithelium showed cloudy swelling and slight fatty changes. Haemorrhages into Bowman's capsule or necrotic lesions, as in rats following the administra-:tion of large doses, were not found.

Protocol of Animal Experiments. III.

Carbon Tetrachloride Toxaemia in Guineapigs.

Animal No.	No. of injec-tions	Total dosage in c.c.	Body wt. in gm.	Size of spleen in c.m.	Spleen wt. in gm.	Estimated normal wt.(1.3 gm.per kilo)	Wt.ratio (enlargement)	Remarks
VII.b.	1	0.1	170	1.7-0.8-0.2	0.24	0.22	1.1	48 hrs
II.a.	1	0.15	195	2.1-0.2-0.25	0.32	0.25	1.3	48 hrs
III.a.	1	0.15	200	1.8-1.0-0.2	0.28	0.26	1.1	
V.b.	1	0.15	260	2.1-1.2-0.25	0.39	0.35	1.1	
VII.a.	1	0.15	260	2.0-1.0-0.2	0.28	0.35	0.80	
VI.a.	1	0.15	400	2.4-1.5-0.3	0.51	0.52	0.98	4 days
II.b.	1	0.2	230	1.8-1.0-0.2	0.32	0.30	1.1	2 days
XII.a.	1	0.2	260	2.5-1.5-0.3	0.55	0.35	1.6	
IV.a.	1	0.25	290	2.5-1.3-0.3	0.53	0.38	1.4	3 days
V.a.	2	0.25	270	1.8-0.8-0.3	0.39	0.36	1.1	10 days
VIII.a.	2	0.25	250	2.0-1.0-0.2	0.32	0.33	0.97	10 days
X.a.	2	0.25	250	1.8-1.0-0.3	0.31	0.33	0.90	10 days
I.a.	2	0.3	190	1.8-1.0-0.2	0.27	0.25	1.1	10 days
XI.a.	2	0.3	260	3.1-1.5-0.25	1.06	0.35	3.0	6 days
XI.b.	2	0.35	410	2.4-1.3-0.2	0.54	0.53	1.0	6 days
X.b.	2	0.35	390	2.0-1.0-0.3	0.33	0.50	0.66	10 days
IX.a.	2	0.35	320	2.5-1.2-0.3	0.45	0.42	1.1	10 days
III.b.	2	0.35	315	2.5-1.3-0.3	0.47	0.42	1.1	12 days

Summary of the Effects of Carbon Tetrachloride Toxaemia in Rats and Guineapigs.

1. In acute carbon tetrachloride toxaemia in rats there is a diffuse syncytial necrosis as well as lymphorrhexis in the spleen. The necrotic reaction is occasionally localised to the marginal zone of the malpighian follicles. The liver at this stage shows acute liver necrosis somewhat like acute yellow atrophy.

2. In chronic toxaemia in rats there is a marked proliferative reaction of the spleen with the formation of free cells from the fixed reticulum. There is a corresponding fibrillary overgrowth which is however not so marked as in manganese toxaemia. The condition of the liver at this stage is one of collapse sclerosis following irregular central necrosis of the lobules.

3. A marked splenomegaly to three or four times the normal size is often present in the early "pre-cirrhotic" stage.

4. With the development of liver cirrhosis after 30-50 injections, there is a change in type of the splenic reaction due to the superadded congestive factor. Distension of the sinuses, the pulp veins and trabecular veins are marked at this stage.

5. In guineapigs the toxic effects of small doses are very marked and result in the production of a free macrophage tissue in the spleen. Necrotic follicle reactions are not very marked.

III. THE TOXIC EFFECTS OF SENECIONINE IN RATS.

Review of the Literature.

The toxic effect following the ingestion of various species of flowering plants belonging to the genus Senecio have been brought into prominence from the incidence of cirrhosis of the liver in cattle fed on grazing grounds contaminated with the weed. The con-:dition was noted in Canada under the name of "Pictou" disease of cattle, in New Zealand as "Winton" disease and in South Africa as "Molteno" disease. It was not unknown in Europe where it went under the name of "Sira-:syke" of Norwegian cattle. Occasionally horses are affected, the name "Dunsiekte" being used for this yawning and staggering sickness of South Africa. Outbreaks have also been described in man (Wilmont and Robertson, 1920; Steyn, 1936) in South Africa due to contamination of wheat by the seed of Senecio.

Of the various species that are widely distributed, so far S. jacobaea, S. burchelli and S. latifolius have been commonly implicated and the opinion has been expressed that S. vulgaris the common "groundsel" of Scotland and England is probably harmless. Further work has shown that the toxicity is due to the small proportion of alkaloids present in the weed. Various alkaloids have been isolated as for example senecionine and/

and senecine from S. vulgaris and S. jacobaea (Grandval and Lajoux, 1895), senecifoline and senecifolidine from S. latifolius (Watt, 1909), fuchsi-senecionine from S. fuchsi and silvasenecine from S. sylvaticus (Müller, 1924), retrorsine from the South African species S. retrorsus, jacobine from S. jacobaea (Menske, 1931), platyphylline and seneciphylline from S. platyphyllus (Orechoff, 1935), squalidine from S. squalidus (Barger and Blackie, 1936), isatidine from S. isatideus (Blackie, 1937). The chemistry of the alkaloids has been investigated by Barger and Blackie (1936). Blackie (1937) divides them into two groups, one completely soluble in chloroform and the other that is also partly water-soluble.

The ingestion of the weed is followed after a variable time by cessation of breeding, a diminished secretion of milk, a disinclination for food and the gradual onset of diarrhoea. The coat becomes dry, the diarrhoea increases and severe straining is followed by prolapse of the rectum. Muscular irrita-:bility is followed by a deepening coma ending in death in a few days. In horses yawning and gaping are early symptoms and are followed by a gradual muscular weak-:ness and inability to walk. The post-mortem appear-:ances have been investigated by numerous workers (Adami, 1902; Johnston; Theiler, 1919, 1920). The condition/

condition of the liver varies from extreme congestion of the portal tracts to a portal and intracellular cirrhosis. The abdominal lymph glands are enlarged, acute ulcers are sometimes met with in the stomach, and ascites and subserous petechiae are frequent.

The aetiological relationship was definitely established by the feeding experiments on cattle by Gilruth (1902) in New Zealand, by Chase (1904) in South Africa, and by Pethick (1906) in Canada. Experimen-:tally the effect of the alkaloids in producing liver damage was first worked out by Cushney (1910-1911) who produced haemorrhagic lesions and necrosis of the liver in frogs, rats, cats and rabbits by subcutaneous injec-:tions of small doses of the alkaloids while larger doses produced pronounced nervous symptoms. Davidson (1935) working with the alkaloid retrorsine isolated from S. retrorsus has established that cirrhosis of the liver develops in rats after repeated subcutaneous injections of the alkaloid, that in the earlier stages there is a well marked endothelial proliferation of the lining of the hepatic venules and that this is followed by rupture and haemorrhage into the central zone. Besides lesions in the liver, he found conges-:tion of the mesenteric nodes, subserous petechiae in the stomach and the mesentery and an enlargement and congestion of the spleen. In similar investigations on/

on frogs, mice, and guineapigs, Chen, Chen and Rose (1935) found that retrorsine in large doses produced pronounced nervous symptoms such as convulsions and coma, while with smaller doses degenerative changes and necrosis were met with, in the liver and kidney.

Material and Methods.

Twenty albino rats averaging about 170 gm in weight were used for these experiments and for controls eight animals that were used as normal standards for studying the effect of carbon tetrachloride were similarly used. One gramme of senecionine was kindly supplied for the investigation by Dr. J.J. Blackie who had extracted it from S. vulgaris. This was dissolved in 100 c.c. of distilled water which was slightly acidulated with acetic acid. This gave a 1% solution containing 10 milligrammes of the alkaloid in 1 c.c. of water. The dose varied from 2 to 5 mg. for studying the effect of small doses and from 5 to 20 mg. for the study of the acute lesions. The injections were given into the subcutaneous tissue of the abdominal wall near the middle line every third day. After the large doses the animals were killed when toxic jaundice was noticed; with smaller doses they were killed at fixed intervals. The entire spleen and pieces of the liver and other viscera were removed and dealt with as described/

:cribed in the methods for studying manganese toxaemia.

Results.

General Effects.

After injections of 10 to 20 mg. of senecionine the toxic effect was so marked that some of the animals showed jaundice and passed into a condition of coma. This effect was also noticed sometimes after two injec-:tions of 5 mg. each. Generally with these smaller doses there was a progressive loss of weight, the skin became dry and lustreless and the hair rough and staring; gradually the animals refused to take food and slight jaundice developed. Ascites was not noticeable in this series, but the urine was high coloured and contained bile pigment. On the whole the changes were very similar to those described by Davidson (1935) with retrorsine except that the ten-:dency to jaundice was more marked and the toxicity was marked with smaller doses.

The Effect of Large Doses (10-20 mg.)

In 48 hours after the injection of 10-20 mg. of senecionine there was well marked oedema at the site of injection. Jaundice was a noticeable feature in one animal. The peritoneal cavity contained a little blood-stained fluid. Subserous petechiae were some-:times/

:times met with in the mesentery, the stomach and intestine. The liver showed areas of subcapsular haemorrhage. It was generally dark and purplish in colour, and soft and flabby in consistence. No definite enlargement of the mesenteric nodes could be made out. The spleen was generally enlarged and dark greyish purple in colour. The kidneys, the heart muscle, the adrenal and the pancreas showed no obvious macroscopic changes. The lungs were congested. Microscopically the spleen showed a variable degree of diffuse congestion. The chief changes were however in the pulp syncytium which had undergone extensive necrosis. Owing to the extreme swelling of the pulp syncytium the mesh was almost indistinguishable. The free cells in the pulp were also affected and pyknosis of nuclei and diffuse lymphorrhexis were common (see Fig. 23). The necrotic changes were most marked around the malpighian follicles at the marginal zone. The liver showed extensive areas of haemorrhagic necrosis in cases which showed jaundice. Healthy liver cells were seldom met with and regenerative activity was not in evidence. The necrotic reaction was most marked in the central and middle zones of the lobules while around the portal tracts the hepatic cells still retained the shape though the nuclei showed varying degrees of karyorrhexis. The haemorrhages seemed/

seemed to be more marked towards the centre of the lobules and seemed to be due to rupture of the sinusoids leading into the central vein. As a result irregular blood "lagoons" were formed as described by Davidson (1935); in between were islands of fused and coagulated liver cells. The necrotic change had not only affec-:ted the hepatic cells but the Küpffer cells lining the sinusoids; many of these latter cells showed vary-:ing degrees of pyknosis and degenerative swelling. The liver cells that were not necrotic showed fatty change and hydropic degeneration.

The kidneys showed marked cloudy swelling of the epithelium of the convoluted tubules. Occasionally some of the glomeruli showed irregular swelling of the endothelium of the capillaries.

The heart muscle showed in places a patchy swelling of the muscle fibres and a hyalinisation suggesting "Zenker's degeneration". The lungs showed active hyperaemia and in places small areas of haemorrhage. The mesenteric glands were enlarged and congested and showed microscopically slight degrees of hyperplasia.

The Effect of Repeated Toxic Doses (4-5 mg.).

Following two to three injections of 4 to 5 mg. doses of senecionine some rats showed well marked jaundice, but others appeared to tolerate the dose with/

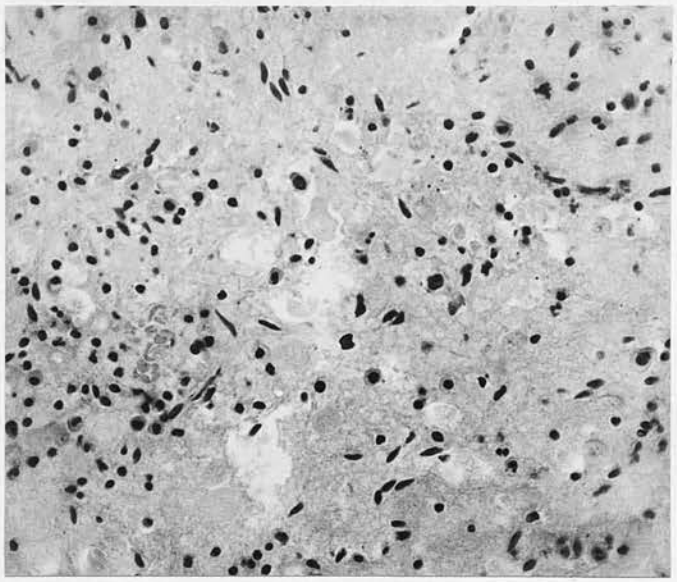


Fig.23. Rat spleen (No.III.3) after a single dose of 10 mg. of senecionine; there is widespread necrosis of the syncytium of the pulp almost like infarcted tissue; the process is diffuse throughout the whole spleen, but not so marked in the lymphoid tissue. (x 350). H and E.

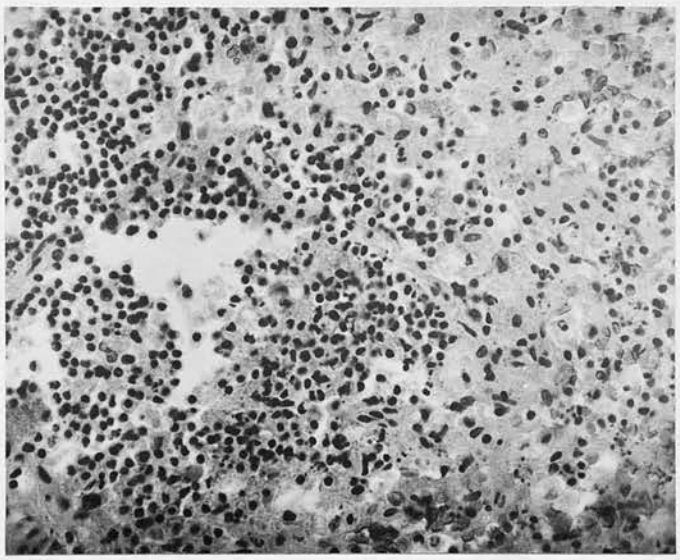


Fig.24. Rat spleen (No.I.4) after 2 injections of 5 mg. of senecionine; necrosis at the marginal zone of the malpighian follicle with lymphorrhexis. (x 300). H and E.

with impunity. At autopsy petechial haemorrhages were met with under the capsule of the liver and subserous coats of the stomach. The spleen was enlarged. The liver was soft and flabby and on section showed small haemorrhagic spots. Microscopically the spleen showed well marked zones of periarterial necrosis at the marginal zone of the malpighian follicles. Around the prefollicular arterioles and the arterial capillary ring separating the marginal zone from the central zones of the follicle there were well marked changes. The syncytial nuclei in this region showed karyolysis and the cytoplasm had become condensed to form a narrow band of tissue. The change was so well marked that the malpighian follicles appeared bordered by well defined white rings of condensed cytoplasm (see Fig.25 and 26) in which the capillary lumen could not be demonstrated. Occasionally haemorrhages were met with into the necrotic areas so that well defined zones of perimalpighian haemorrhage were formed (see Fig.25). Even at these early stages an increase in the reticulum fibres of the follicles could be demonstrated, so that with the Foot-Wilder stain the mesh work of reticulin stood out amidst the necrotic and condensed cytoplasmic mass as a well defined net work which is extremely thin and hardly visible (see Figs.29 and 30). The pulp cords showed well marked shrinkage so that the sinuses appeared/

appeared unduly widened and prominent (see Fig.27). A remarkable cytological change was the alteration in type of syncytial nuclei from plump more or less round oval endothelial types into elongated oval forms very much like the nuclei of the fibroblasts; some were much more condensed and resembled the nuclei of the fibro-:cytes. Along with this differentiation of the syn-:cytium a marked fibrillary increase could be demons-:trated by the Foot-Wilder stain so that the appearance was the formation of numerous venous spaces bounded by condensed and fibrotic pulp cords, a change that is remarkably similar to the appearance of pulp "fibro-adenie" that is typical of the Banti spleen in man (see Figs.27 and 32). Capsulo-trabecular thickening and fibrous spread was variable. The fibrous altera-:tion in the pulp could not be regarded as an extension from the capsule and trabeculae but due to some other factor acting intrinsically on the pulp. The changes in the liver were more or less similar to those des-:cribed by Davidson (1935) with retrorsine. Side by side with necrotic changes affecting the hepatic cell, regenerative activity could also be made out. Irre-:gular sinusoidal haemorrhages were common.

The Effect of Smaller Doses.

After ten to sixteen injections of senecionine in doses gradually increased from 2 to 4 mg. twice and thrice/

thrice weekly the spleen showed well marked enlargement up to three or four times the normal size. The liver was firmer in consistence and slightly more difficult to cut. No coarse cirrhosis could be made out, but in some cases a faint granularity of the surface was noticeable. Histologically the splenic enlargement was found to be due to a remarkable proliferation of the reticulo-endothelium of the marginal zone of the follicles (see Fig.33) as well as of focal areas of the pulp. The lymphoid aggregations in the pulp in the perivascular and peritrabecular zones were much larger in size than normal and had spread out more or less diffusely into the pulp. The pulp cords appeared richer in nuclei. In places focal collections of pale staining syncytial nuclear groups seemed to be the points of activity from which proliferative changes spread to the pulp. Many of these foci were however overrun with lymphocytes and basophilic cells resembling plasma cells suggesting the possibility of a common origin from the syncytium. Congestion was slight and diffuse and there was no suggestion of a stasis in the sinuses. Erythrophagocytosis was more marked than normal. Megakaryocytes appeared more frequent than normal. In the liver there were four well defined changes. Of these, (1) the hepatic cells showed constant regenerative changes side by side with necrosis;/

necrosis; many of the cells had large nuclei often three times the normal size. In some the chromatin was more prominent, in others it was diffuse. Some of the cells appeared much larger than normal. Scattered here and there throughout the lobule were basophilic liver cells with pyknotic nuclei. There was no distinct localisation into definite areas of necrosis but the appearance was that of a diffuse toxic damage followed by remarkable regenerative activity. (2) Another noticeable change was the marked hypertrophy and hyperchromasia of the Kupffer cell system of the sinusoids (see Fig.36). The cells had become oval in shape and had often migrated from their lining walls to form elongated dark staining cells blocking up the sinusoids. (3) The endothelium of the hepatic venules showed a remarkable hyperplastic activity. Often the proliferative change was so marked that masses of oval cells had formed rounded clusters almost filling up the hepatic venules (see Fig.35). (4) Side by side with this proliferative activity the portal tracts showed well marked changes. Numerous lymphoid cells and plasma cells had formed dense clusters around the bile ducts and portal venules, and had formed irregular extensions in between the lobules and more irregularly into the surrounding tissue (see Fig.34). Into these, inflammatory fibro-

:blastic/

:blastic growth had commenced and thin strands of collagen could be seen extending in between lobules in the manner of a portal cirrhosis. In between this newly formed fibrous inflammatory tissue, proliferating bile ductules had also extended irregularly from the portal tracts.

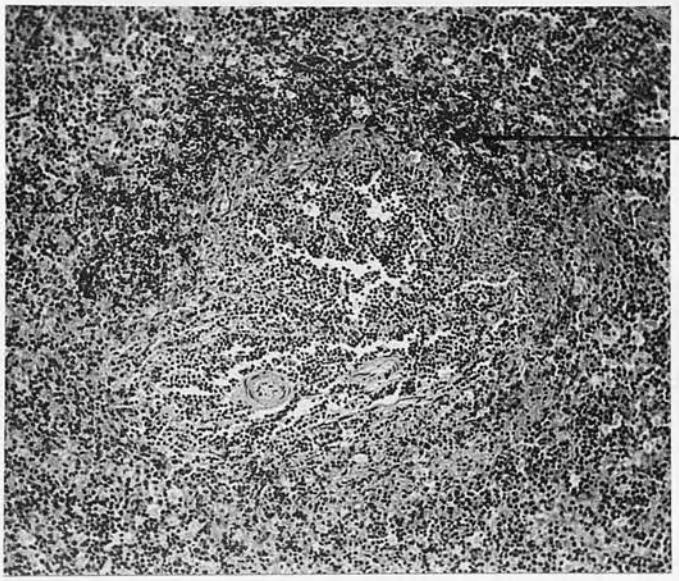


Fig.25. Rat spleen (No.I.4) after two injections of 5 mg. of senecionine showing haemorrhage (H) into a zone of peri-malpighian necrosis. (x 120). H and E.

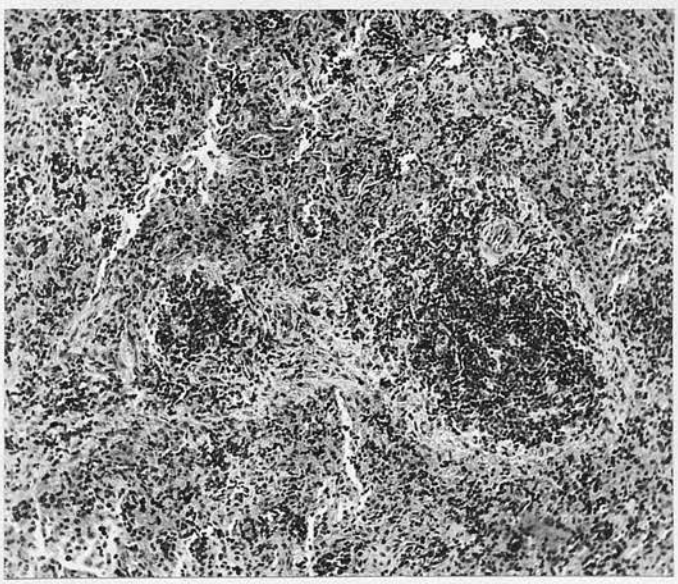


Fig.26. Rat spleen (No.II.2) after 3 injections of 4-5 mg. of senecionine showing typical periarterial fibrosis, peri-malpighian fibrosis, atrophy of the follicle and commencing fibrosis of the pulp. (x 120). H and E.

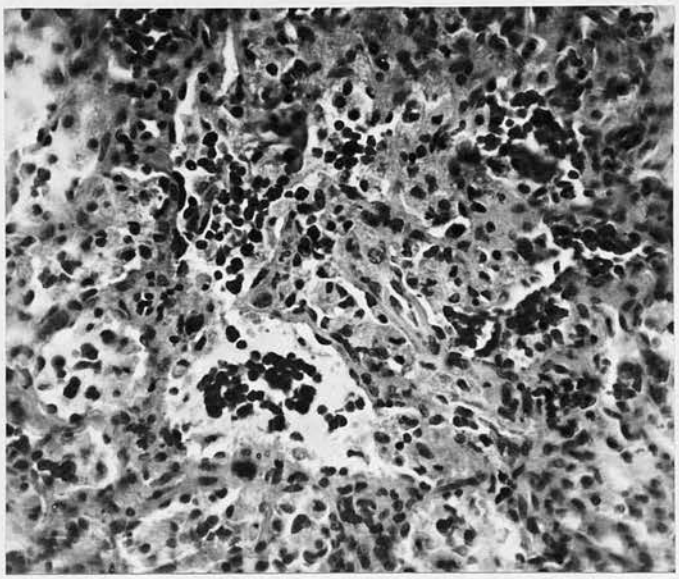


Fig.27. Rat spleen (No.II.2) after 3 injections of 5 mg. of senecionine; fibrosis of the pulp cord with condensation of the reticulum and widening of the sinus, changes similar to "fibro-adenie" of the pulp. (x 350). H and E.

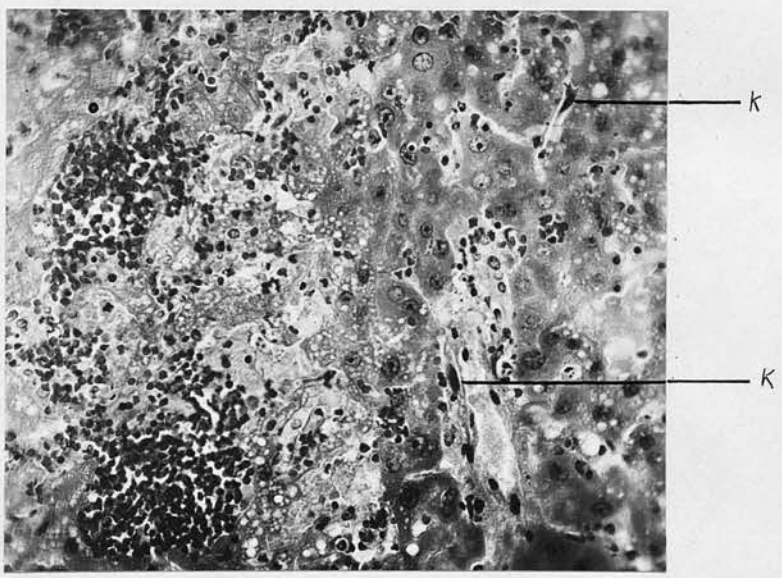


Fig.28. Rat liver (No.I.4) after two injections of 5 mg. doses of senecionine showing the necrosis of the liver cells, haemorrhages from the sinusoids and the swelling of the Küpffer cells (K) and pyknosis of their nuclei. (x 280). H and E.

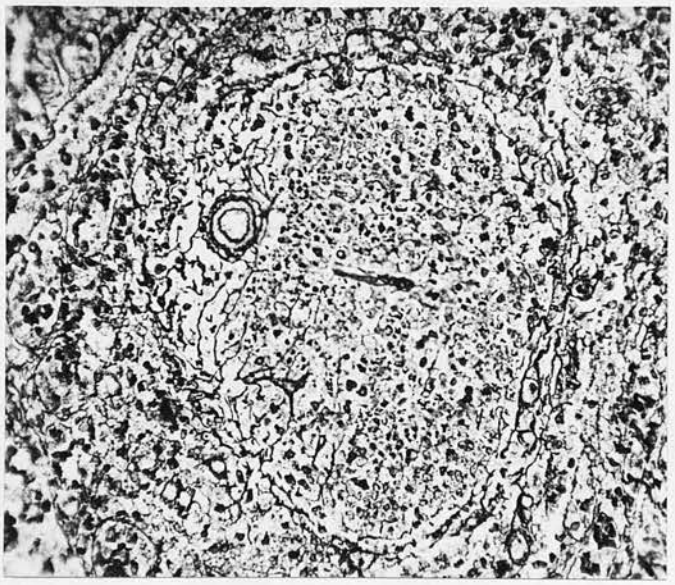


Fig.29. Rat spleen (No.I.4) after two injections of senecionine showing early fibrillary increase at the marginal zone of the follicle. (x 275). Foot-Wilder.

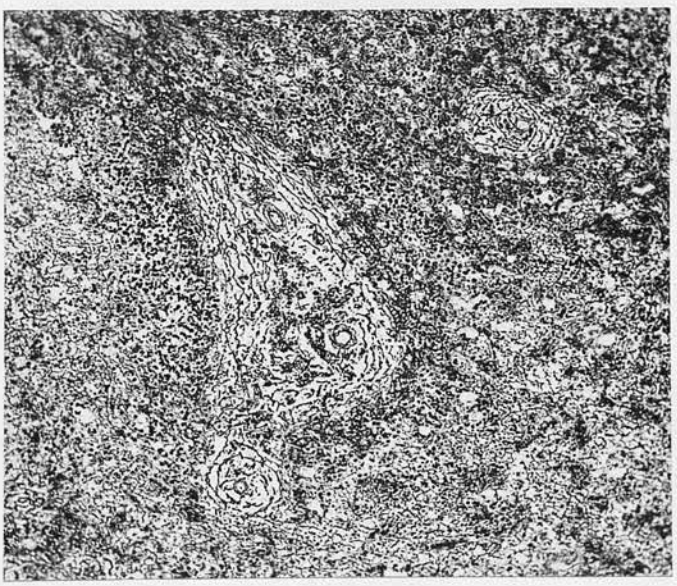


Fig.30. Rat spleen (No.II.3) showing more marked fibrillary increase in the malpighian follicles after 3 injections of senecionine. (x 120). Foot-Wilder.

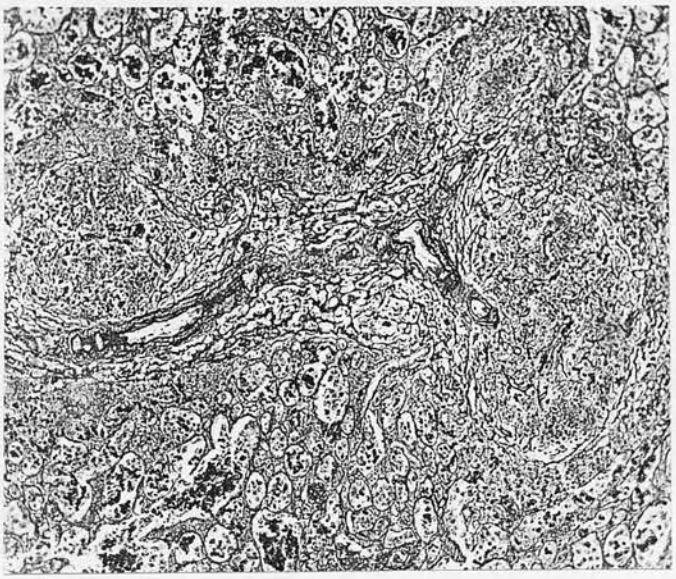


Fig.31. Rat spleen (No.II.3) after 3 injections of senecionine showing typical periarterial fibrosis around the pre-follicular arteriole and spreading fibrillary change in the pulp cords. (x 120). Foot-Wilder.

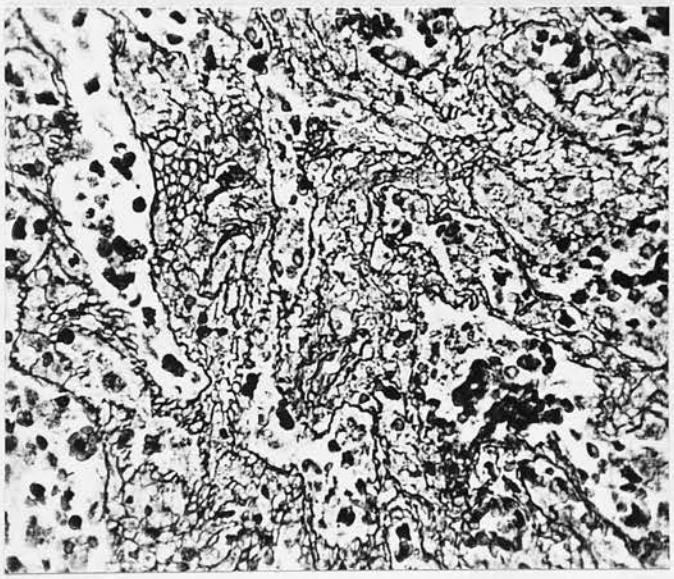


Fig.32. Rat spleen (No.II.3) after 3 injections of senecionine showing typical "fibro-adenie" of the pulp cords. (x 390). Foot-Wilder.

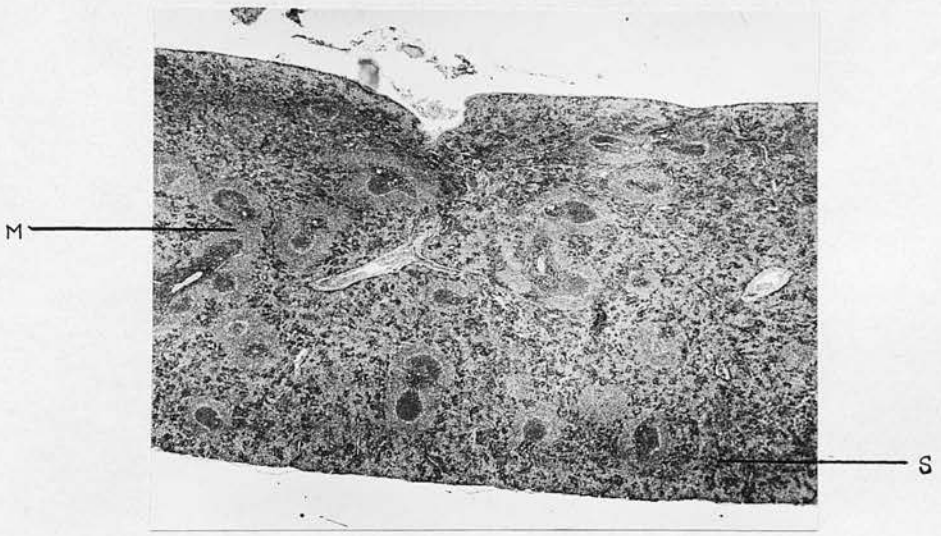


Fig.33. Rat spleen (No.IV.1) after 16 injections of 2-4 mg. doses of senecionine showing the hyperplasia of the marginal zones (M), of the malpighian follicles and secondary lymphoid foci (S) in the pulp. (x 14). H and E.

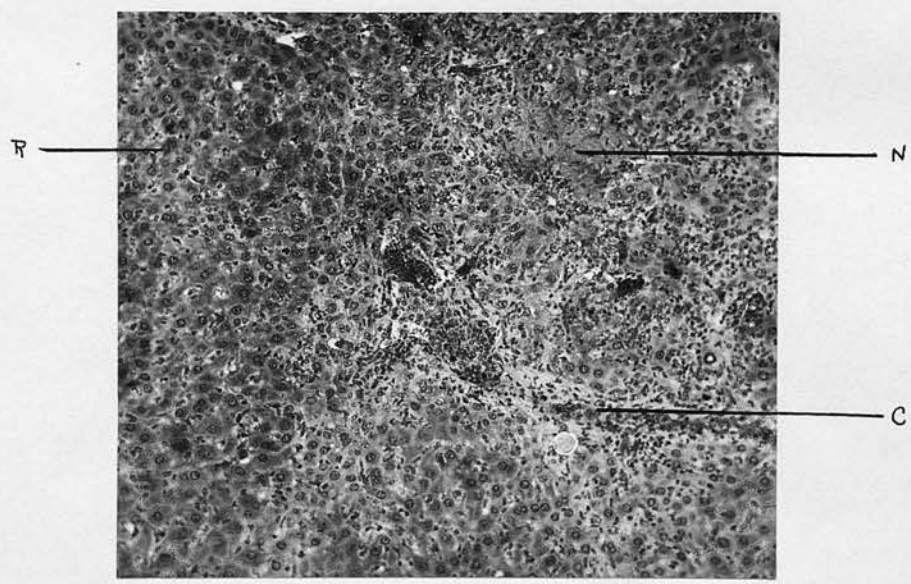


Fig.34. Rat liver (No.IV.2) after 16 injections of 2-4 mg. of senecionine showing periportal infiltration (C), areas of necrosis (N) and areas of regenerative activity (R). (x 120). H and E.

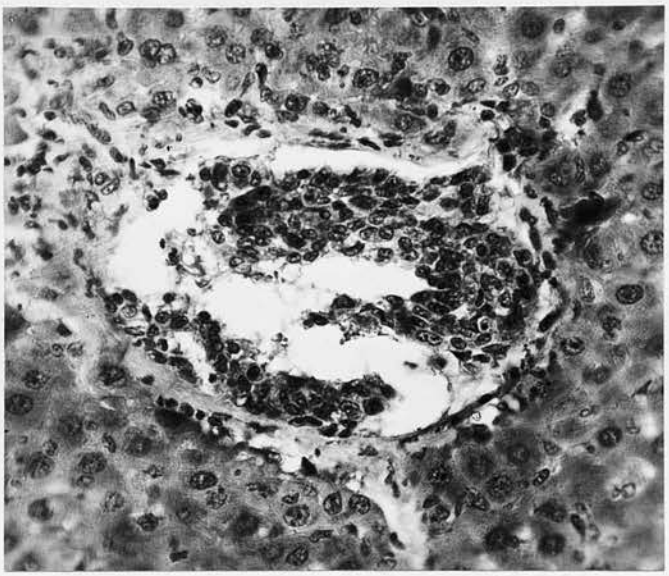


Fig.35. Rat liver (No.II.1) after 16 injections of 3-4 mg. of senecionine showing the marked endo-:thelial proliferation inside a central vein; note that some of the liver cells in the neighbourhood show deep staining pyknotic nuclei. (x 275). H and E.

Fig.36. Rat liver (No.II.1) after 16 injections of senecionine showing the hyperplasia of the Küpffer cells lying in between the columns of liver cells. (x 275). H and E.

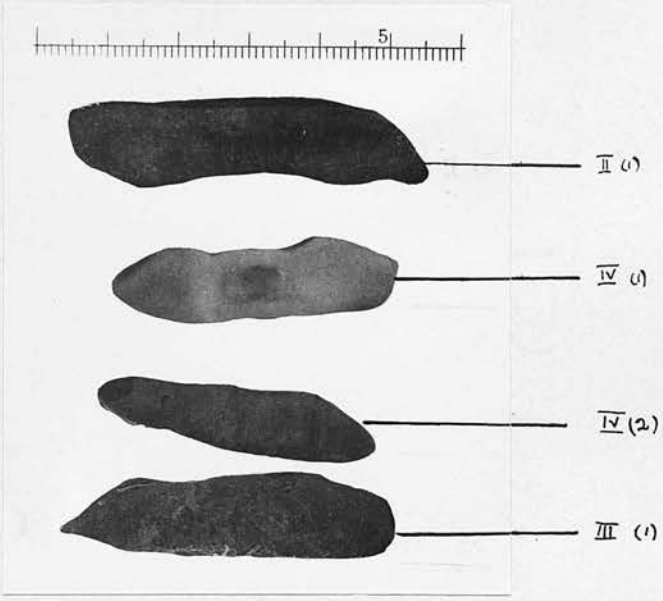


Fig.37. Rat spleen after 16 injections of 4-5 mg. of senecionine showing the marked enlargement.

Fig.38. Spleen of normal rats weighing 150 gm. and 210 gm. shown for comparison.

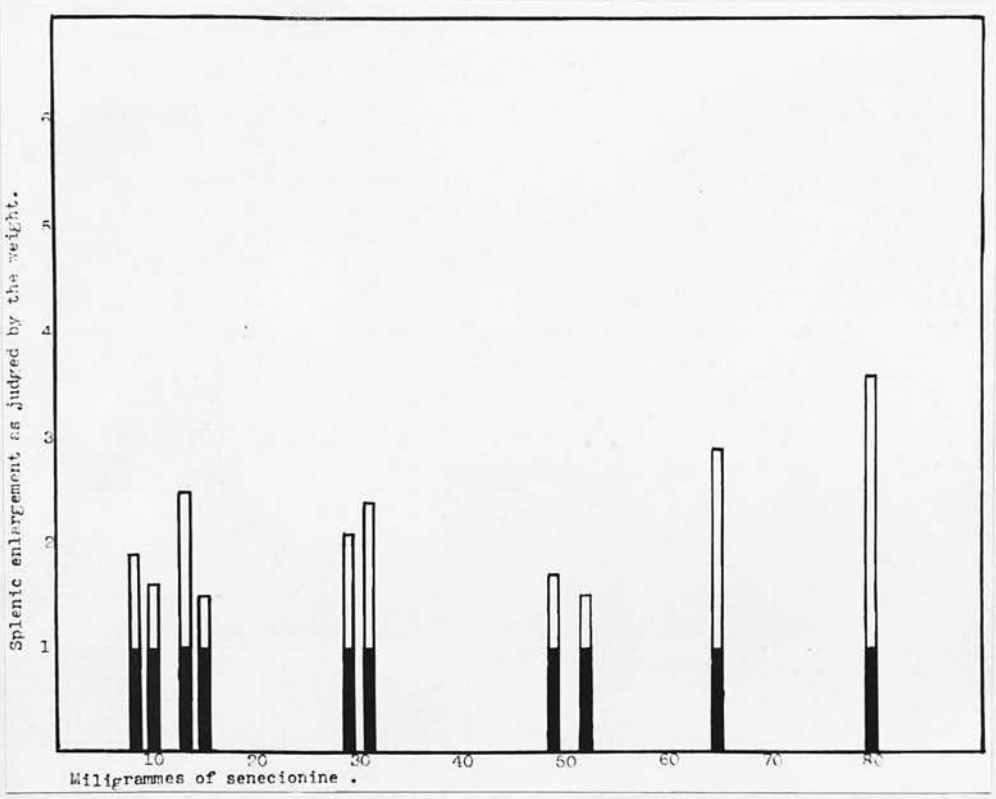
Protocol of Animal Experiments. IV.

Senecionine Toxaemia in Rats.

Animal No.	No. of injections	Body wt. in gm.	Mgm. of alkaloid	Size of spleen in cm.	Spleen wt. in gm.	Estimated normal wt.	Wt. ratio	Remarks
++III.4	1	200	20	4.0-1.2-0.35	1.15	0.54	2.1	Killed 48 hrs.
++III.3	1	250	10	4.0-0.9-0.3	0.98	0.68	1.4	Killed 48 hrs.
II.3	2	145	8	4.0-0.8-0.3	0.88	0.39	2.3	Jaundice
II.4	2	140	8	3.5-0.6-0.25	0.53	0.38	1.4	
IV.3	2	160	8	4.0-0.7-0.25	0.64	0.43	1.5	
IV.4	2	150	8	3.8-0.9-0.3	0.89	0.41	2.2	Jaundice
I.4	2	140	10	3.4-0.9-0.3	0.60	0.38	1.6	
II.2	3	135	13	3.5-1.0-0.35	0.94	0.37	2.5	Marked jaundice
I.3	3	180	15	3.5-0.7-0.3	0.74	0.49	1.5	Slight jaundice
I.2	3	150	15	3.1-0.6-0.25	0.59	0.41	1.4	
V.4	10	170	29	3.7-1.0-0.3	0.95	0.46	2.1	
V.3	10	210	31	4.2-1.2-0.3	1.45	0.57	2.5	
V.2	10	190	31	3.7-1.0-0.3	1.10	0.51	2.2	
III.2	9	210	49	4.0-0.9-0.35	0.98	0.57	1.7	
I.1	12	190	52	3.2-0.7-0.3	0.75	0.51	1.5	
II.1	16	300	65	5.0-1.3-0.5	2.25	0.81	2.8	
IV.2	16	190	65	4.0-1.2-0.35	1.55	0.51	3.0	
IV.1	16	210	65	4.2-1.3-0.4	1.65	0.57	2.9	
III.1	16	210	80	5.0-1.3-0.5	2.05	0.57	3.6	
+V.1	6	140	11	3.5-1.0-0.3	0.75	0.38	2.6	

+ shows the effect of repeated small doses of less than 2 mg.

++ shows the effect of single large doses of 10 mg.



Histogram III showing the splenic enlargement after repeated injections of senecionine. The black columns represent estimated normal spleen weights.

Summary of the Effects of Senecionine Toxaemia in Rats.

1. In acute toxaemia induced by injections of senecionine in rats there is syncytial necrosis especially of the marginal zone of the malpighian follicles of the spleen. In the liver, necrosis around the hepatic veins and sinusoidal haemorrhages are produced.

2. In subacute toxaemia with senecionine there is a collapse sclerosis and fibrillary overgrowth in the periarterial zone of the malpighian follicles followed by a gradual fibrillary increase in the pulp, reactions which correspond to the "fibro-adenie" of the malpighian follicles and the pulp cords as met with in Banti's disease.

3. With repeated smaller doses there is a marked splenomegaly of about four times the normal size due to hyperplastic changes affecting the reticulo-endo-:thelium of the pulp. Scattered foci of reticulum are activated and differentiated to form clusters of free mononuclear cells with intermingled lymphoid cells and plasma cells. In the liver there is a marked endothelial proliferation of the hepatic veins, hyper-:plastic changes in the Küpffer cells and early portal cirrhosis.

DISCUSSION.

A Toxic Splenomegaly.

An analysis of the tables showing the earlier stages of toxic action demonstrates that splenic enlargement occurs with all the three cirrhogenic agents quite early and before the cirrhosis develops. The splenomegaly is thus pre-cirrhotic in that it is present in the early stages of liver necrosis. A consideration of human pathology will show that similar splenic enlargements have been described in the morbid anatomy of acute and subacute liver atrophy, but have been looked upon as "reactive" or "congestive" in nature and their real significance has not been appre-:ciated. These experiments on rabbits, rats and guineapigs have shown that degenerative changes in the spleen are common but are not so well defined owing to the diffuse structure of the syncytium in contrast to the glandular structure of the liver. Just as patterns of liver necrosis arise from the special mode of action of the toxic agent, there is a splenic pattern of necrosis due to the tendency for localisation at the marginal zone of the malpighian follicles where the arterial capillary system opens out into the pulp. A perivascular distribution of the lesion is often met with, and with senecionine and manganese the necrotic reaction can be seen to affect the arterial wall and its/

its lining endothelium. While some follicles are completely destroyed others show only partial lesions. Throughout the stages of toxic action, regenerative reactions can be made out in the follicles, and with smaller doses and in the stage of recovery from toxic action these changes are well marked. There is thus experimental evidence of a type of splenomegaly that is due to a simultaneous effect of the toxic agent on the spleen and the liver. Further, it has been demonstrated that in the experimental animal types of cirrhosis morphologically similar to portal and biliary cirrhosis in man are associated with this type of toxic splenomegaly. In the later stages of the type of portal cirrhosis produced by carbon tetrachloride in rats, there is the change in type of the splenic reaction from a congestive factor due to portal stasis.

"Fibro-adenie".

The development of the Banti lesion (Banti, 1910) of "fibro-adenie" of the malpighian follicles as a reaction to repeated peri-arterial reticular necrosis in the experimental animal following toxic damage, suggests that the lesion is only the prototype of an inflammatory fibrosis that is commonly met with in other organs in various inflammatory conditions. It differs from other types of toxic necrosis in that the lesion/

lesion is typically peri-vascular unless toxic damage is extreme. It differs also from replacement fibrosis in that fibroblastic growth from the vessel wall is slight, and secondary; a collapse sclerosis is followed by a collagenous transformation of the reticulum fibrils as well as increased overgrowth of the reticulum. Many lymphocytes are destroyed in the necrotic process and in the later stages the follicles gradually become acellular and fibrous. The change is most marked around the penicillar arterioles as well as the large pre-follicular arterioles where the "fibro-adenie" can be seen as a demilune of fibrous tissue on one side of the follicle or in between neighbouring follicles. The distribution of the lesion and the morphology of the fully developed stages are exactly similar to what Banti (1910) described as the characteristic feature of Morbus Banti in man though he denied the inflammatory nature of the disease process. With senecionine in rats the"fibro-adenic" process is confined not only to the follicles, but is widespread in the pulp so that the cords of Billroth become altered to fibrous bands which show elongated oval nuclei some pyknotic and degenerate and others enlarged and active, suggesting repeated damage.

The Significance of Haemorrhages in the Spleen.

The arterial and periarterial necrosis and its association/

association with focal areas of haemorrhage into the perimalpighian zone after two and three injections of senecionine, raise questions of great importance. Such an acute haemorrhagic lesion cannot obviously be regarded as due to chronic venous stasis followed by rupture. On the other hand, there is a severe toxic necrosis of the capillary walls at the marginal zones where the arterial capillaries open out into the pulp. The haemorrhage can only be regarded as due to a leak-:age of blood through the damaged endothelium of the vessel wall. In acute carbon tetrachloride poisoning in man, the occurrence of gastric haemorrhage and petechiae in other organs can be better correlated with the toxic effect of the drug rather than any venous stasis. The siderotic nodule and splenic haemorrhages in man have all been looked upon as the result of mechanical effects of the circulation, so that the possibility of a necrotizing lesion that may cause a vascular leakage has not been sufficiently stressed. In any case, periarterial necrosis and occasional haemorrhage into the necrotic zone are so well defined in experimental senecionine poisoning that the possi-:bility of a similar mechanism in splenic anaemia cannot be dismissed. McMichael (1931) has stressed that the fibrotic periarterial lesions in splenic anaemia contain blood and that the fibrosis is the result

result of previous haemorrhage followed by organisa-:tion. However, the present study indicates that this need not necessarily be the case, as the haemorrhage may be the result of a toxic vascular lesion in a necrotic patch.

Hyperplastic Reactions.

The presence of extreme hypertrophy of the mal-:pighian follicles in cases where regeneration had been allowed to take place by a prolonged period of rest (6 weeks), after repeated follicle damage, is another feature of great interest. The hyperplastic cells of the follicle with their clear-staining nuclei and scanty protoplasm and general ovoid shape resemble endothelial cells formed by differentiation from the cytoplasmic reticulum. The changes were most marked around the central capillary mesh-work of the follicle and also at the marginal zone where the capillary mesh is even more abundant (see diagram I of the circulation of the malpighian follicle). The process seemed to be one of alteration of the lymphoid mesh-work into a germ-centre type of cell from which it is assumed that the lymphocytes arise. The wide-spread proliferative reaction with enlargement of the follicles to three or four times the normal diameter and the alteration to a more embryonic cell type, offer many features of resem-:blance to the condition described in man under the names/

names of "giant lymph follicle hyperplasia" (Brill, Baehr and Rosenthal, 1925) and "lymphoid reticulosis" (Ross, 1933), conditions which are regarded as simple (Ross, 1933) or neoplastic (McNee, 1934) types of reticulo-endothelioses. The production of such reactions in rabbits and rats after repeated follicle damage suggests the possibility that the conditions met with in man are similar regenerative lymphoid reactions following chronic toxic damage. In one case of lymphoid reticulosis in man that was studied during the course of this investigation, the hyper-:trophied follicles showed in places large collections of haemosiderin pigment in haematophages at the mar-:ginal zone, a reaction that is quite common in manganese toxaemia.

Another type of proliferative reaction that was met with was not specially related to the malpighian follicles, but was more diffuse and involved the secon-:dary lymphoid foci in the pulp. Here small foci of reticulo-endothelium seemed to be activated and assumed proliferative growth. This effect was particularly marked with repeated small doses of senecionine. In two cases this reaction was so marked after carbon tetrachloride injections that on examination of the spleen the histological picture resembled that of a monocytic leukaemia; however, the other organs showed no/

no similar infiltrations. It would thus appear that as a result of a chronic stimulus proliferative reac-:tions, sometimes affecting the lymphoid tissue and sometimes the reticulo-endothelium, are met with in the experimental animal. Further, these reactions offer many features of resemblance to the differing types of "reticulo-endotheliosses" or "reticulosis" that have been described in man.

A Splenic Anaemia Syndrome.

The histological evidence of a marked phagocytic destruction of the red blood cells in senecionine, manganese and carbon tetrachloride toxaemias in experi-:mental animals suggests that there is the stimulation of a phagocytic mechanism in the spleen. Such a stimulation is not only to abnormal or foreign cells in the circulation, but may occur as an exaggeration of the normal function owing to an infective or toxic stimulus. Thus while Addison (1919) has demonstrated that after the injection of washed pigeon's corpuscles into the rabbit's blood there is the development of a phagocytic activity in the spleen, a similar mechanism has also been described in the rabbit's spleen after infections (Muir, 1902). With regard to the mechanism of the destruction Rous (1923) has demonstrated that in the normal spleen whole corpuscles may be ingested by the haematophages while ingestion may also occur after fragmentation/

fragmentation of the erythrocyte. In the present study of toxic reactions in the spleen, there is definite histological evidence of ingestion of whole cells rather than an extra cellular fragmentation, suggesting that there is a hyperactivity of the haema-:tophages. The studies of Takada (1932) have shown that after repeated injections of carbon tetrachloride there is the development of an anaemia in older animals and a reticulocytosis in the young. There is thus conclusive evidence of the development of a splenic anaemia, which is however not a primary splenic dis-:order, but only the morbid expression of a general toxic process where the spleen shows an exaggeration of a normal function. If one were to compare the histological picture to the condition of splenic anaemia in man, called Bengal splenomegaly (Part II, Chapter I) the similarity is striking in the presence of giant erythrophagocytes in the pulp and in the evidence of a chronic splenic inflammation.

The Pathogenesis of the Lienal Fibrosis and Proliferation.

In regard to the mode of development of hepatic cirrhosis Cameron and Karunaratne (1936) have advanced a theory that is of special interest in its analogy to the pathogenesis of the splenic lesion. They suggest that it is the product of autolysis of the hepatic cells/

cells drained by the lymph spaces to the portal tracts, that stimulates fibroblastic growth, that in the earlier stages, liver destruction is compensated by regeneration of the liver cells until a stage is reached when such regeneration is inadequate. If we now turn to the histological picture in the spleen during the toxic reaction we find the early development of a periarter-:ial necrosis, provided the dosage has been sufficient to affect the more resistant mesenchymal tissue. As in liver cirrhosis while the lesions induced by smaller doses are compensated by regenerative proliferation, with repeated toxic damage there is a tendency for the persistance of the lesion with gradual spread of fibro-:blastic tissue from the periarterial zone. Histolo-:gical studies have shown that thus there is a combina-:tion of a collapse sclerosis followed by proliferative fibrosis.

It seems therefore that there is an essential similarity in the splenic reaction to the process of repeated destruction and repair in hepatic cirrhosis. It is true however that the necrotic reactions are much more distinct and histologically evident in the specia-:lised hepatic cells than in the primitive tissue of the spleen.

The effect of a smaller dose in producing prolif-:erative growth of splenic tissue is also a factor that requires explanation. It seems probable that a pro-:cess of repeated focal damage is followed by the liberation/

liberation of growth promoting substances which act on the cells of the spleen rather than a direct irritative growth induced by the toxic agent. The presence of reticulo-endothelial stimulants capable of inducing proliferative reactions is well known with extracts of splenic tissue. While there has been no definite agreement with regard to the mode of action of these extracts (see Danilewosky, 1895; Pearce, Krumbhaar and Frazier, 1917; Eddy, 1921; Leake and Leake, 1923 and 1924) all are agreed that after injection they induce increased haemopoietic activity. Louros (1928) in a study of the reticulo-endothelial activity of various substances has found that spleen extract and spleen lipoid are the most potent. A theory to account for the proliferative reaction would be that as a result of repeated irritative action or toxic damage the forma-:tion of these growth promoting substances in the spleen is stimulated. Evidence for the consequent hyperacti-:vity of the spleen is shown not only by an increase of splenic tissue, but by the remarkable erythrophagocytic activity that is usually present. Further, in some cases this destructive effect on the red cells is some-:times compensated by an overproduction in the bone marrow as demonstrated by Smyth and Smyth (1936) in carbon tetrachloride toxaemia in rats.

General Summary.

1. It has been shown that during the stages of chronic toxaemia induced in animals by manganese chloride and the alkaloid senecionine, definite lesions are produced in the marginal zones of the malpighian follicles and in the pulp cords. These consist of necrosis of the pulp syncytium followed by collapse and overgrowth of the fibrillary reticulum and colla-:genisation. These changes are most marked around the small arterioles; further, there is an extension of fibrous tissue from the adventitia of the vessels into the affected areas. The lesions thus produced are more or less similar to the periarterial fibrosis and fibrosis of the pulp which Banti described under the term "fibro-adenie".

2. During the development of these changes there is an increased erythrophagocytic activity in the spleen and a splenomegaly which is quite marked in the early stages, and is thus independent of the hepatic cirrhosis which is produced in the liver in the late stage.

3. With another cirrhogenic agent carbon tetra-:chloride, follicle reactions are not quite so distinct, but there is a marked proliferative reaction with the formation of numerous mononuclear cells in the spleen during the early stages of liver damage. In the later stages,/

stages, with the development of portal cirrhosis in the liver, there is a superadded congestive reaction in the spleen.

4. Hyperplastic reactions in the malpighian follicles are found with very small doses; such reactions bear a resemblance to the condition des-:cribed as "lymphoid reticulosis" in man.

5. There is thus experimental evidence that during the stages of the development of liver cirrhosis both of a portal and a biliary type there are fibrotic and proliferative reactions in the spleen and a definite splenomegaly which is not dependent in the early stages, on portal stasis. There is also presumptive evidence of a splenic anaemia during the toxaemic process.

ACKNOWLEDGEMENT:

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PATHOLOGICAL STUDIES ON SPLENOMEGALY

PART I. EXPERIMENTAL STUDIES

CHAPTER III.

THE SPLENIC REACTION IN SIMIAN MALARIA.

THE SPLENIC REACTION IN SIMIAN MALARIA.

INTRODUCTION.

The occurrence of malarial plasmodia in various species of anthropoid apes, chimpanzees and monkeys first attracted attention in the search for a possible animal reservoir of human malaria. None of the species met with however were identified with human plasmodia. Attempts have also been made in the ex-:perimental infection of monkeys with human plasmodia, with variable results. The trend of recent work has been to study the mechanism of immunity in monkey malaria. Problems such as the resistance to super-:infection, the degree of reticulo-endothelial response as judged by the cytological changes in the blood, and the effect of splenectomy or a "blockade" of the spleen in lowering the resistance have all been exten-:sively studied. Simian malaria has also been used in the evaluation of different therapeutic agents as plasmodicides, but comparatively little work has been done in the study of the pathological lesions and their possible correlation with the lesions of human malaria. No excuse is therefore needed if the study of the behaviour of the reticulo-endothelial system in monkey malaria is considered as a prelude to the study of the splenic/

splenic reaction in human malaria.

With regard to suitable plasmodial species for purposes of study the one described by Koch (1898) and named after him Plasmodium kochi produces only mild infections and is therefore unsuitable for study of the lesions. Another species that has also been studied is Plasmodium reichenowi, a form indistinguish-:able from P. vivax and found in chimpanzees (Reichenow, 1917) in the Cameroons. In America Taliaferro (1932) has used the quartan parasite Plasmodium brasilianum for his experimental work. In India, a species dis-:covered in a macacus monkey by Napier and Campbell (1932) and named Plasmodium knowlesi (Sinton and Mulligan, 1932) has many advantages for experimental work, since it is easily available, and produces a severe infection with most of the clinical features of hyperacute malaria, such as enlargement of the spleen, remittant fever, progressive anaemia, great prostration, and in 30 to 60 per cent of cases haema-:globinuria. The infection is almost invariably fatal in the common rhesus monkey. Treatment with quinine may help to tide over the crisis and render the condition subchronic, but relapses occur. This infection can easily be transmitted by direct inocu-:lation or by a Bass culture. While various species such as Silenus irus, Silenus radiatus, Silenus cynomologus, Semnopichecus entellus are all capable

of/

of being experimentally infected, Silenus rhesus is the species that is extremely susceptible (Knowles and Das Gupta, 1932). The infection is generally fatal in about a week after the appearance of parasites in the blood. It was hoped that this severe form of fulminant malaria might afford a pathological parallel to the severe form of malignant malaria met with in man, due to infection with Plasmodium falciparum. The effect of a toxic factor would show itself, if present, in the type of the lesions met with.

Of previous pathological studies Taliaferro (1932) has drawn attention to the macrophage activity, the general lymphoid hyperplasia in the spleen, and the tissue localisations met with in monkey malaria. Row, Dalal and Gollerkeri (1933) have noted the enor-:mous clusters of parasites and pigment met with in the spleen and the other organs. They describe the condition of the spleen as one of acute red infarction, caused by the occlusion of parasitic plugs in the vessels. Knowles and Das Gupta (1932) have studied in great detail the various stages of the development of the parasites in the blood with regard to Plasmodium knowlesi, while Taliaferro and Taliaferro (1934) have dealt with Plasmodium brasilianum. The haematological changes in the blood have also been studied (Malamos, 1934; Denecke and Malamos, 1935; Krishnan/

Krishnan, Lal and Napier, 1933). The effect of splenectomy in inducing haemaglobinuria has been described by Krishnan and Ghosh (1935). Taliaferro and Cannon (1934) have indicated that the splenic reaction varies with the stage of the infection, in that phagocytosis in the spleen takes place only during the crisis and not before.

MATERIAL AND METHODS OF STUDY.

The material that was studied was obtained from twelve monkeys of the species Macacus (Silenus) rhesus infected with the Kasauli strain of Plasmodium knowlesi. Five of these specimens were obtained from Major Wats, I.M.S., of the Malarial Survey of India, and the other seven were obtained from Dr. R. Row of the Haffkine Institute, Bombay. Nine of the specimens were Zenker fixed and subsequently preserved in 70 per cent alcohol when they were brought from India. Nine of these were post-fixed, after washing in water for 48 hours, in Helly's fluid and sections cut after paraffin em-:bedding. Staining was carried out by the following methods:- 1. Mayer's haemalum and eosine; 2. Mallory's aniline blue; 3. Wilder's modification of Foot's stain; 4. Wolbach's method of staining with Giemsa; 5. Turn-:bull's modification of Jenner's stain for tissues; 6. Perle's Berlin blue reaction. A few frozen sections were stained with Sudan III and some were tried again for the Berlin blue reaction. It was found in practice that the best method for demonstra-:tion of parasites was by Jenner's stain for tissues which was found to give better results than the Giemsa or Giemsa-Leishman stains. For reticulum, Wilder's modification of Foot's stain was found the most convenient of all the Foot-Beilschowsky methods. In five/

five of these specimens, only the spleens were studied, but in others some of the other organs were examined in order to assess the degree of reticulo-endothelial and vascular response as compared with the changes in the spleen. In three cases only slides were examined. Three spleens of healthy monkeys were studied as controls.

History of infected monkeys.

Monkey No.27.

Inoculated from monkey No.O.S.1.K4 on 21-10-35, Parasites appeared on 27-10-35. ++ Rings. Died on 1-11-35. Passed blackwater.

Monkey No.28.

Inoculated from monkey No.O.S.I.K4 on 21-10-35. Parasites appeared on 4-11-35. ++ Rings. Died at 12.30 p.m. on 4-11-35.

Monkey No.A.42.

Inoculated from monkey No.O.S.I.K4 on 21-10-35. Parasites appeared on 27-10-35. ++ Rings. Died on 30-10-35. Passed blackwater.

Monkey No.A.61.

Inoculated from monkey No.318.K4 on 23-6-36. Parasites appeared on 25-6-36. ++ Rings. Died on 2-7-36. Passed blackwater.

Monkey No.A.63.

Inoculated from monkey No.318.K4 on 25-6-36. Parasites appeared on 26-6-36. Schiz. Died on 1-7-36. Passed blackwater.

Monkey/

HISTOLOGICAL CHANGES IN SPLEEN.

Specimen No.	Oedema. Capsule & Trabecular.	Pulp Congestion	Follicle Hyperplasia	Necrosis		Endophlebitis Hyperplastic	Sinus Reaction	R.E. Differen-:tiation and Phagocytosis.	Syncytial Degeneration.	Pale Nuclear Proliferation.	Phagocytosis of Plasmodia.	Adhesion of Parasites to Syncytium.	Type of Reaction, Other Changes.
				Central	Annular								
27	Slight	++	++	+	++	+++	++	++	Nil	Nil	++	+	Acute
28	Slight	++	++	+	+	+++	+++	++	+	Nil	++	+	Acute
A.61	+	++	++	+	+	++	+	+	Nil	Nil	+	Nil	Acute
A.63	+++	+	Nil		+	+	+	+	+++	Nil	+	++	Acute
A.42	++	+	Nil	+	+	+	+	+	+++	Nil	+	+	Acute
K.1	Nil	++	+	-	-	++	?	+	++	Nil	?	+++	Acute
K.3	++	?	Nil	-	-	?	+	+	+	Nil	?	++	Acute
K.5	+	?	Nil	-	-	+	Slight	+	Nil	Nil	+	+++	Acute; miliary tubercles
K.6	++	Slight	++	?	-	+	+	+	Nil	++	+	-	Sub-acute reaction.
K.7	Slight	+	+	+	-	+	+	++	Nil	Slight	+	+	Acute
K.8	+	+	Nil	+	-	+	+	++	Slight	Nil	?	++	Acute
K.2 hronic)	+	-	+	+	-	+	+	+	Nil	+++	?	-	Sub-chronic reaction

Monkey No.K1. Silenus rhesus,Aet. 18 months.

Inoculated intraperitoneally with three drops from Pithecus pilatus 55 (Kasauli) 24-9-32. Few rings 27-9-32; large number of rings 29-9-32. Died 1-10-32. P.M. done 6 hours after.

Monkey K.2. Silenus rhesus, aet. 18 months.

Inoculated with blood from K.1. 29-9-32. ++ rings 3-10-32. Quinine 2 gr. per os 4-10-32. Repeated 6-10-32, parasites fewer; absent 7-10-32; rings 11-10-32. Quinine repeated 13-10-32, parasites absent from 16-10-32 to 24-10-32; + rings 25-10-32; parasites absent 28-10-32; animal quite well, a few gametocytes 10-11-32; a few gametocytes but quite well 10-12-32. Killed.

Monkey No.K.3. Silenus rhesus, aet. 2 years.

Inoculated with blood from K.2. on 10-12-32. + rings and gametocytes 16-10-32. Given 2 gr. quinine 19-10-32. Found right arm paralysed; moribund; killed 22-10-32.

Monkey K.5. Silenus sinicus, aet. 8 months.

Inoculated with blood of K.4. 25-10-32. ++ rings 29-10-32; diarrhoea 30-10-32. Died 1-11-32. P.M. showed tubercles in peritoneum.

Monkey K.6. Silenus rhesus, aet. 4 years.

Inoculated with blood of K.2. 28-10-32. ++ rings 1-11-32. Quinine given 4-11-32 to 5-11-32. Re-

:covered/

:covered but rings still present; gametocytes 11-11-32. Died of hydraemia 13-12-32. Infection controlled for two months.

Monkey K.7. Silenus sinicus.

Inoculated with blood of K.4. No other records available.

Monkey K.8. Silenus sinicus; aet. 4 years.

Inoculated with blood of K.7. 4-11-32. ++ rings 10-11-32, blood loaded with rings 12-11-32. Quinine given; moribund 13-11-32. Killed with chloroform 13-11-32.

Macroscopic features.

The enlargement varied from twice to three times the normal. The capsule was tense and stretched, dull and of a slate grey colour. It was easily torn and in many cases, the dark colour of the pulp could be seen through. In some cases distension was so marked that the organ felt like a tense bladder. On section, the colour varied from a dark greyish brown, to a tarry black. The cut surface was bulging and the pulp generally soft. In the fixed specimens, the malpighian bodies appeared as greyish white spots. The trabecular markings were indistinct.

Microscopic features.

The chief changes were met with in the meshes of the/

the pulp and the venous sinuses.

The capsulo-trabecular system showed the effect of acute distension of the organ. The capsule was often thin and stretched, the normal wavy wrinkling was lost, and there was comparatively little compen-:satory thickening. Slight perisplenitis was shown by the swelling and desquamation of the serosa and the presence of fibrinous threads. An actual capsulitis with cell infiltration was not met with, but sometimes the collagen bundles were separated by oedema. The trabeculae were widely separated and extended as straight thin bands into the medulla where thicker branches could be made out. There was no increased trabecular branching. In a few cases the trabecular bundles were swollen by oedema and the muscular and collagen bundles split up. Necrotic changes affecting the nuclei were met with only in one instance. There was no increase of muscle or elastic tissue. Peri-:trabecular infiltrations were present usually around all the smaller branches. The cells were mostly of the lymphoid series, but plasma cells and mononuclear cells were also present. The veins of the trabeculae, showed changes that could be regarded as significant, since they were found more or less constant in all the twelve specimens studied. These veins, which are the continuations of the pulp veins which drain the venous/

venous sinuses, showed a characteristic type of hyper-:plastic endophlebitis. The endothelial cells were often raised up by collections of plasma cells and lymphocytes three or four layers in thickness. The endothelial lining was often split into layers, many cells becoming rounded and ovoid while some were cast off as free phagocytes into the lumen. The trabecular arterioles often showed perivascular extensions of their lymphoid sheaths into the trabeculae from the follicles.

The malpighian bodies were generally large well-developed, numerous and discrete. Well marked pro-:liferating germ-centres were found in 6 of this series. The definition of the marginal zones was variable; they were distinct in 5 of these specimens. Peri-:malpighian haemorrhage was not met with, but a zone of congestion was present round the follicles in many cases though it could not be sharply demarcated from the general congestion of the pulp. A striking feature was the presence of necrosis in the follicles sometimes occurring in the centre, but more often in a zone at the periphery. The change seemed to affect the reticulum cells just within the marginal zone pro-:bably around the capillary mesh in this region. The marginal necrosis seemed to affect the protoplasm of the cells in this region as a diffuse hyaline trans-:formation with swelling of the cytoplasm and karyo-:lysis/

:lysis of the nuclei. Some of the small capillaries in this region showed parasitic plugs and occasionally the lumen could not be demonstrated, owing to endo-:thelial swelling. The staining reactions of amyloid substance were not present. In some cases only central necrosis of the follicle was present. The large eccentric artery of the tuft showed no intimal hyaline, but some swelling of the endothelium was present in the smaller vessels. In two cases the appearance suggested a necrosis of the periarterial sheaths which are normally well developed in the monkey. The cells of the follicle were mostly small lymphocytes, but in active follicles numerous large lymphocytes predominated. Round proliferating germ centres, large lymphoblastic cells corresponding to the type I described by Hu (1934), with basophilic protoplasm, large nuclei with large acidophilic nucleoli were present. Many follicles were in the resting phase. Plasma cells with basophilic cytoplasm were present at the marginal zone, but these cells have been normally found to occur in the monkey. Apart from an occasional eosinophile, other cells were not met with. The eccentric arterioles of the tuft showed in some specimens well marked penicillar branching which seemed to take place within the node in the monkey. The larger arterioles showed no degenerative changes within the lumen except slight splitting/

splitting of the intima. The penicillar sheaths described by Billroth (1861, 1862), and Schweigger-Seidel (1862, 1863), could occasionally be demonstrated. The endothelial lining of the smaller arterioles could often be seen projecting into the lumen as irregular knobs or rod-shaped protusions. In some cases slits were present in these adventitial sheaths and these occasionally contained blood. No endarteritis was present. In specimen A.42 the arterioles of the node were very much dilated and appeared in places distended by masses of parasites and blood cells within the lumen so that an actual blockage of the vessel was regarded as possible. No actual thrombosis with fibrin formation could be demonstrated. The appearance was exactly similar to that met with in the brain in "cerebral malaria" in man where the cerebral capillaries appear stuffed with parasites. The appearance of necrosis of some of the perivascular sheaths of the smaller branches suggested a relative circulatory stasis.

The venous sinuses of the pulp were clearly defined in 6 of the specimens and were collapsed in all the others, though they could be made out on careful examination under the capsule. The longitudinal bands which formed the walls showed no thickening. These normally appear as coarser structures than ordinary/

Fig.I. Spleen No.27 (x 80) showing the trabecular vein with hyperplastic endophlebitis and the trabecular artery with a lymphoid sheath (Haemalum and Eosin).

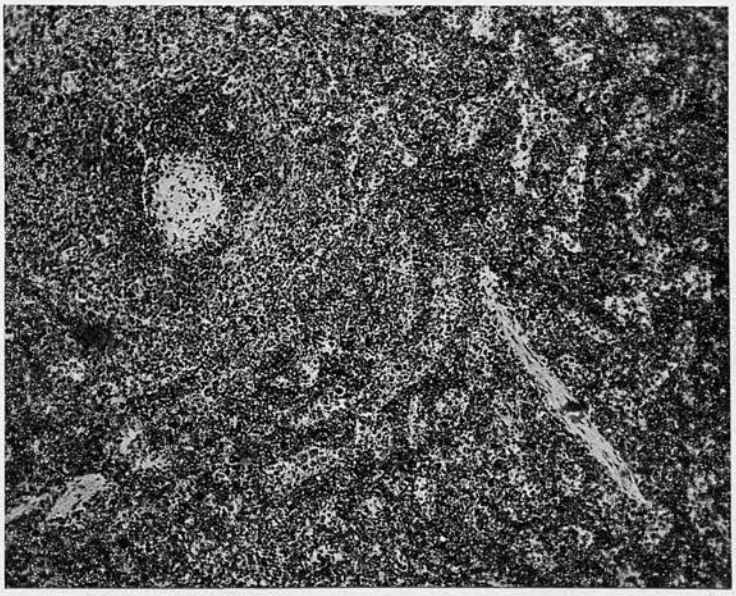

Fig.II. Spleen No.27 (x 80) showing the widening of the sinuses and engorgement of the pulp cords with necrosis in a malpighian body. (Haemalum and Eosin).

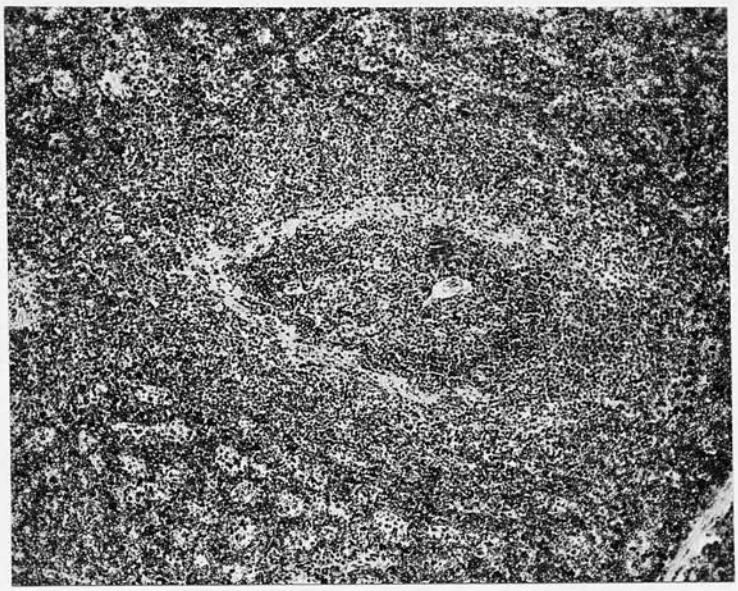

Fig.III. Spleen No.27 (x 80) showing a ring of hyalinisation and necrosis round the capillary network at the periphery of the follicle. (Haemalum and Eosin).

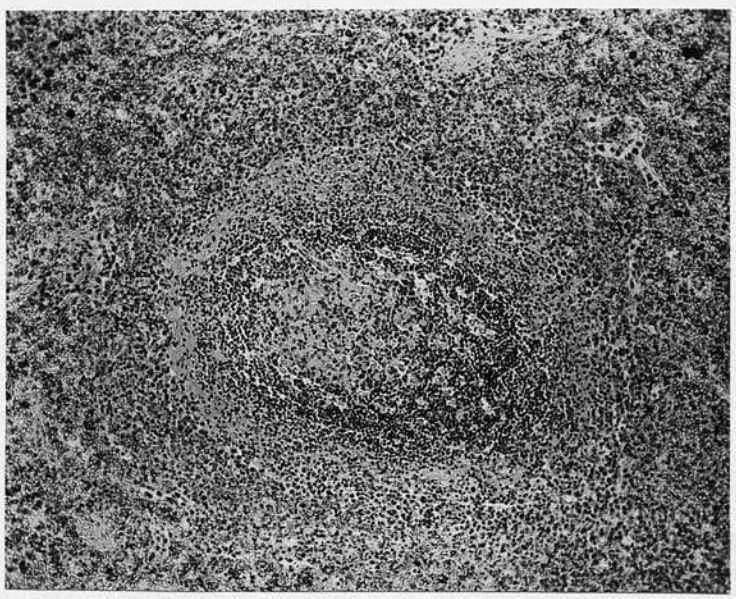

Fig.IV. Spleen No.28 (x 80) showing ring of necrosis in the marginal zone and irregular hyalinisation in the centre. (Haemalum and Eosin).

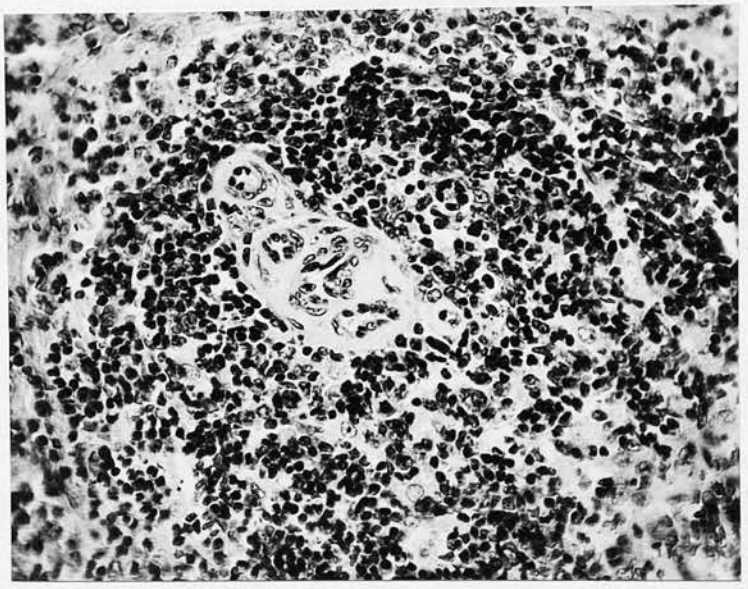

Fig. V. Spleen (350) showing the penicillar branching of the sheathed artery of the follicle. (Haemalum and Eosin).

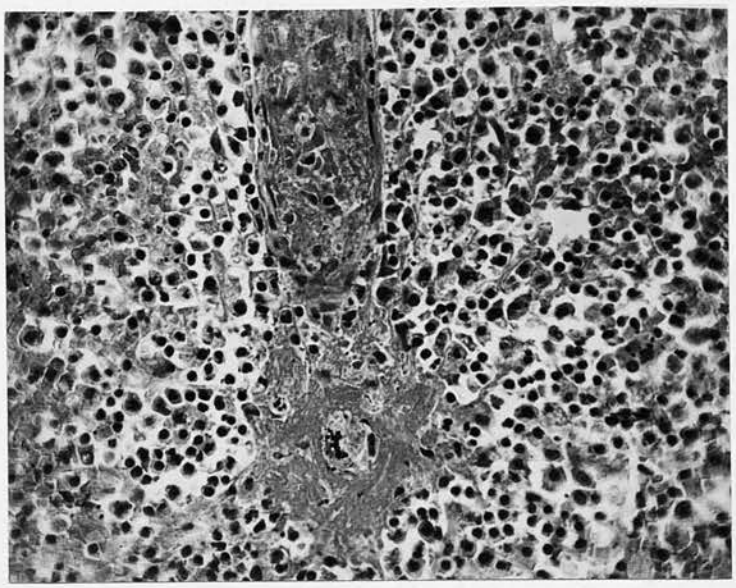

Fig.VI. Spleen A.42 (x 350) showing a dilated malpighian artery filled with a mass of parasites and blood cells: note the hyalinisa-:tion and necrosis of the sheath just below. (Jenner).

Fig.VII. Spleen A.42 (x 800) showing the pulp reaction with free phagocytic histiocytes and the degenerative swelling and necrosis affecting the cytoplasmic reticulum. (Jenner).

ordinary reticulum (Foot, 1927) while Weidenreich (1901) holds that they are not fibres at all, but longitudinal bands of tissue somewhat like the staves of a barrel bound together by encircling hoops of reticulum. The lining endothelium was often flat or ovoid with plump nuclei, but often the cells pro-:jected into the lumen. In many cases a lack of continuity of the endothelial lining suggested that the sinuses were in communication with the vascular spaces by irregular slits, the stomata of Mollier (Mollier, 1910). Proliferative activity of the sinus cells was marked, not so much by the duplication of cell layers, but by the occurrence of rounded cells which were set free into the lumen. All stages of transition between plump lining cells projecting into the lumen, irregular amoeboid cells still attached, and cells getting separated could be demonstrated. Many of the littoral cells showed phagocytosis of parasites and pigment, even before they were set free into the lumen. The free cells were often rounded sometimes irregular in shape had a faintly staining round nucleus where the nuclear chromatin was poorly developed. Stained with Jenner's stain they could not be distinguished from similar cells met with in the pulp which were probably tissue histiocytes derived from the cytoplasmic reticulum. They were markedly phagocytic and were loaded with granules and irregular/

irregular clumps of malarial pigment and often para-:sites. In many cases the cytoplasm was vacuolated and the nuclear staining obscured by the collections of pigment. With Jenner's stain phagocytosis of parasites was well made out, each small cluster of pigment being surrounded by a faint blue plasmodial mass. Often the cytoplasm had disintegrated leaving the pigment in a vacuole inside the enclosing cell. Stages of digestion of pigment could also be made out, as a brown haze around a cluster of granules. Some-:times the cell appeared outlined by a faint rim of pigment and the cytoplasm was so vacuolated and the nucleus disintegrated and necrotic, that the pigment alone persisted in a shadow cell. More often the ingested pigment appeared as coccoid granules, rodlets, bars or even faint dust-like granules barely visible, while from repeated accumulation in specimen K.11 dense irregular clusters and blocks, looking like irregular lumps of coal, were met with. Erythro-:phagocytosis was well marked. Many of the ingested red cells contained parasites. Many red blood cells were present in the sinuses. The congestion was not however so marked as in venous stasis. Many of these red blood cells appeared to be infected with plasmodia while some appeared to be disintegrating. Other free cells in the sinuses were mostly of the lymphoid series, the/

the small lymphocytes being the most numerous. Cells of the lymphoblastic type were rarely met with. Polymorphonuclear cells did not appear in larger numbers than in the blood stream. Eosinophiles were sometimes met with, and occasionally plasma cells were present.

The cords of Billroth show marked engorgement except in specimen K.2. which showed a subchronic type of reaction. The engorgement was mostly due to the accumulation of blood within the meshes of the pulp. Often the swelling of the pulp cords caused com-:pression and collapse of the sinuses. The red cells are mostly irregular in shape,, partly from the effects of fixation and possibly from haemolysis. A large proportion of the red blood cells showed small clusters of pigment which when stained with Jenner or Giemsa showed various stages of parasitic development. Many of the cells appeared adhering to the reticular mesh, while some were actually phagocytosed by the primitive reticular syncytium.

The cytoplasmic reticular syncytium showed oedema and cloudy swelling which could not be regarded as an autolytic change. The branching threads were often swollen and broken up and the nuclear staining was poor in many specimens indicating a degenerative cloudy swelling. Proliferative changes were present, but slight, many of the nuclei becoming ovoid, rounded or irregularly/

irregularly oval in shape. Some cells appeared as large free phagocytes in the pulp mesh. Stages indicative of differentiation into cells of the histio-:cyte series could be made out. During this change the nucleus gradually became rounded, the nuclear membrane less convoluted, and the peripheral arrange-:ment of chromatin less distinct. These free cells of the reticulum were most actively phagocytic to parasites and pigment granules and in later stages appeared as large irregular cells completely encrusted with irregular masses of pigment. Even the nuclear outline and the cytoplasm became indistinguishable. Erythrophagocytosis and vacuolation of the cytoplasm were also met with as in the littoral phagocytes. Mitotic figures in the reticulum could hardly be made out, indicating a lack of any marked hyperplastic reaction. Other free cells met with in the pulp were more or less similar to those in the sinuses. Mega-:karyocytes were rare. Normoblasts and erythroblasts were also infrequent. Abnormal leucocytes of the myeloid series were hardly ever met with.

The fibrillary reticulum of the pulp showed no increase in sections stained by the Foot-Wilder method. There was no increase in the periarterial zones. Peritrabecular increase was also not in evidence.

The parasites were mostly dividing forms of Plasmodium knowlesi. Ring forms, half grown forms and/

and full grown forms were also numerous. Gametocytes could not be very well distinguished in the sections. Plasmodial cytoplasm was very well defined by Jenner's stain and the various stages of plasmodial phagocytosis could be studied. The adhesion of free parasites to the syncytial mesh was well marked. Most of the parasites were intracorpuscular but occasionally the cytoplasm of the red cell could not be made out. Knowles and Das Gupta (1932) have described this feature as characteristic of the growing trophzoit. Parasitic chromatin was not sharply demarcated, but occasionally the typical double masses on ring forms could be made out.

The pigment that was present, was brownish black haemozoin which could be dissolved out by ammonium sulphide and other alkalies, and did not give the Berlin blue reaction of haemosiderin. In size, the granules varied, but coccoid forms about 1 micra in diameter were most numerous and were probably central clusters after schizogony. Occasionally finer rodlets, bars or irregular clumps could be demonstrated inside parasites and red blood cells. Inside macrophages the pigment had accumulated to form dense clusters and often finer grains. Recently ingested granules had a vacuole all round where the parasitic cytoplasm had disintegrated. Often the adventitial cells around the vessels showed a longitudinal stippling with pigment/

pigment and sometimes the reticulum cells were so outlined. The distribution and the intensity of accumulation was of some significance. It was most marked in the pulp cords either in the free histio-:cytes or in the fixed reticulum cells or inside parasites in the syncytial mesh. To a less extent, it was found in the venous sinuses and in their lining cells, while the malpighian bodies showed least. Here it was almost entirely confined to the reticulum cells in the follicles, and to a slight extent around the vessels in the perivascular adven-:titial cells. No evidence of phagocytosis of pigment was shown by the lining endothelium of arterioles or penicilli. Occasionally the trabeculae and the capsule showed a few granules on the surface, but there was no evidence of engulfment. Haemo-:siderin was relatively scanty, but as the specimens were Zenker fixed this was of doubtful significance.

In specimen K.5. with a coexisting tuberculous infection the tubercle follicles showed up as rela-:tively pale areas almost free from pigment. Cunning-:ham, Sabin, Sugiyama and Kinwald (1925) and Sabin, Doan and Forkner (1930) have described the epitheloid cells of the tubercle follicle as derived from the large mononuclear cells, the bacilli being found ingested by these cells. The effect of such an ingestion in curtailing the functional activity of these/

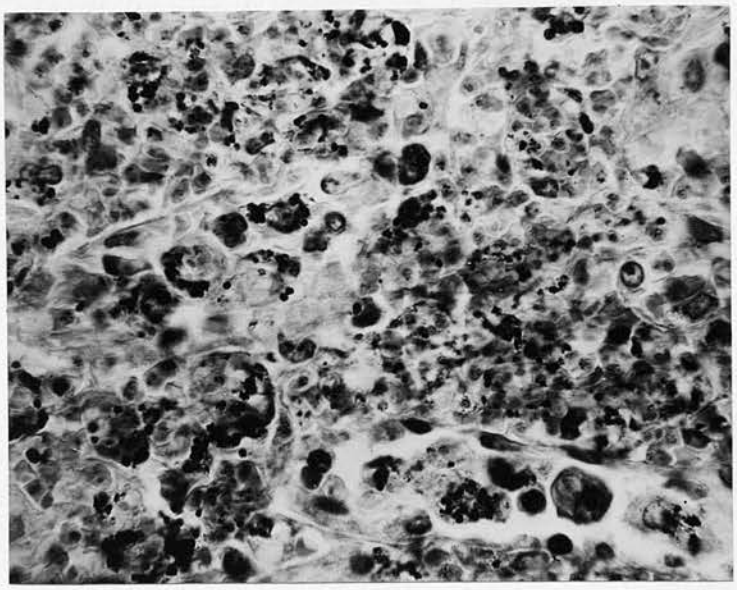

Fig.VIII. Spleen 28 (x 850) showing the concentration of parasites in two Billroth cords and the phago-:cytic reaction in the sinus. (Jenner).

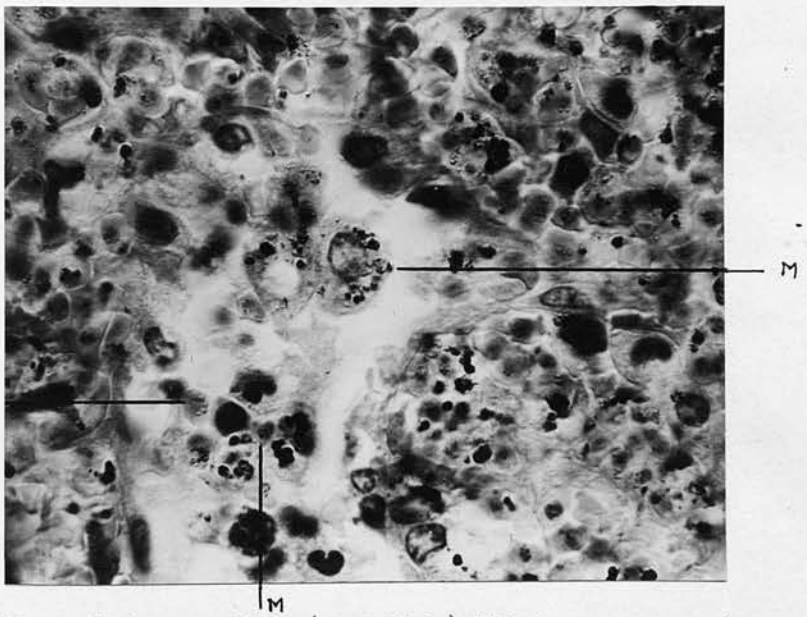

Fig.IX. Spleen 28. (x 1000) High power view of a sinus showing the phagocytosis of rings, half grown forms, and dividing forms and the vacuola-:tion of the macrophages, (M). (Jenner).

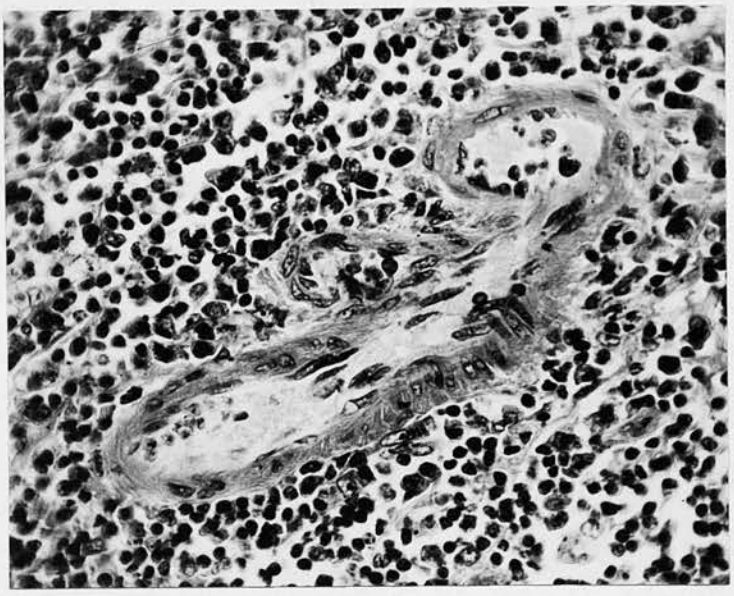

Fig.X. Spleen 28. (x 400) showing parasites within the eccentric arteriole. (Haemalum and Eosin).

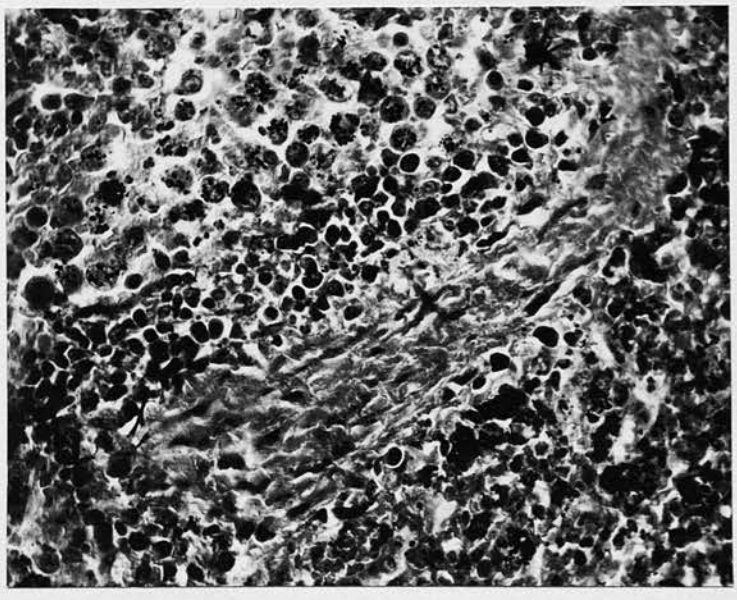

Fig.XI. Spleen 27. (x 400) showing peritrabecular infiltration with plasma cells and lymphocytes. (Haemalum and Eosin).

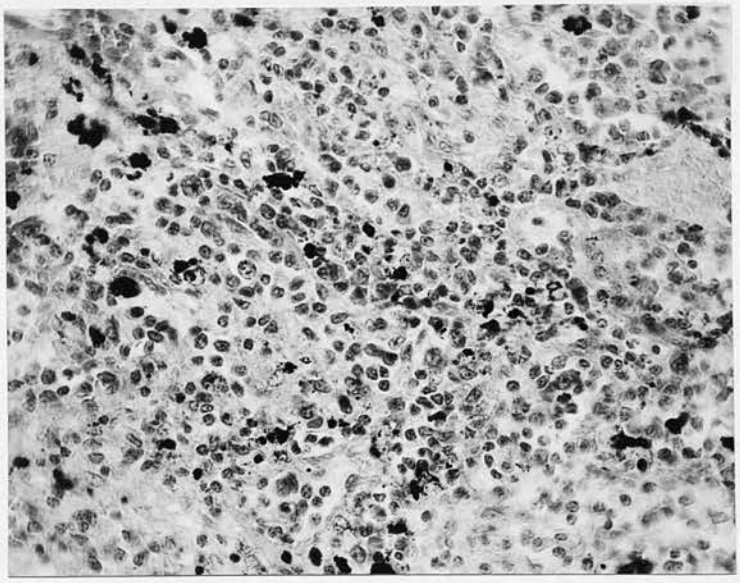

Fig.XII. Spleen K.2.. (x 350) showing "pale nuclear proliferation" a subchronic reaction. Note the presence of dense clusters of pigment indicating old infection. (Haemalum and Eosin).

Fig.XIII. Liver K.5. (x 350) showing phagocytosis by free Küpffer cells in the sinuses as well as the fatty deganeration and cloudy swelling and poor nuclear staining of the liver cells. (Mallory's aniline blue).

these cells is shown by their altered behaviour to malarial pigment. A state of "blockage" is presum-:ably established.

The Liver.

The histopathological changes met with in the liver in the six specimens studied were as follows:- Capsular thickening and subcapsular infiltration were absent except in one case where tuberculosis was a complication. There was no round celled infiltration round the portal tracts. The portal veins were generally distended and parasites were numerous in the lumen. Some were inside large macrophages probably swept on from the splenic vein. A few leucocytes and lymphoid cells were also present. No endophlebitis of the portal venules could be made out. The branches of the bile duct were healthy. The hepatic arterioles showed slight swelling of the endothelium and numerous parasites were found in the lumen. The central veins were relatively empty, except for a few parasites present inside. The sinusoidal capillaries were mostly dilated but there was not much blood in the lumen. Parasites were found in numbers, but many appeared phagocytosed by the Kùpffer cells. Some of these were loaded with pigment granules and parasites and appeared as sausage shaped bladder-like cells free in the lumen. In one case, K.2. with a subchronic reaction/

reaction in the spleen, free parasites appeared scanty but the Küpffer cells showed dense pigment masses indicating an old infection. Hyperplastic changes affecting the reticulo-endothelium were slight. No areas of focal necrosis of the liver cells could be made out, but the liver cell columns showed cloudy swelling and in some cases fatty degeneration. One case showed marked intercellular oedema and another glycogen storage in excess. Haemosiderin was well marked inside the liver cells.

The Adrenals.

The histopathological changes in the adrenals consisted in the presence of marked parasitic collec-:tions in the capillaries both of the cortex and of the medulla. Hyaline capillary thrombi or parasitic plugs causing actual occlusion were not met with. The concentration of parasites was most marked in the zona reticulata and the zona fascicularis. In one case the appearance suggested a special localisation in the adrenal, but capillary haemorrhages from actual blockage were not met with. Small areas of necrosis were occasionally found in the zona reticulata. These appeared as rather diffuse areas where the cells showed no nuclear staining. Calcification was well marked in one case and very slight in another. The cortical cells showed extensive foamy degeneration. Phagocytic activity of the lining reticulo-endothelium

was/

was present in every case, but was not very marked as compared with that in the liver and spleen. It is interesting to note that none of these cases showed basophilic granules described by Natali (1934).

The Kidney.

The kidneys were examined in three cases. All showed parasites in the glomerular capillaries. Proliferative glomerulitis was not present, Slight adhesion to Bowman's capsule was noticed in one case. The capillaries in between the tubules showed numerous parasites. Degenerative cloudy swelling of the tubular epithelium was well marked in all the cases and in one case well marked fatty degeneration was present. Here there were casts in the lumen. One case showed coexisting miliary tubercles.

The Heart Muscle.

The capillaries in between the muscular bundles showed parasites in all the three cases. The arterioles showed similar collections. In one case, the parasites were so numerous that they appeared like chains of cocci in the small vessels in between the muscle bundles. Some appeared to be ingested by the lining endothelium. In places, parasites appeared to lie actually in the muscle fibres as described by Gaskell and Millar (1920) in human malaria, but on careful examination these were found to be in the lumen/

lumen of small vessels in between the undifferentiated protoplasmic fibres. Inflammatory changes were not met with in any of these cases. The muscle fibres were atrophied in two cases and fatty degeneration was marked in one.

The Bone Marrow.

The bone marrow was studied only in one case. It showed a typical erythroblastic reaction with the extension of erythroblastic tissue into the fatty marrow. Microscopically normoblasts and erythroblasts were numerous. Myeloblasts were common but granular myelocytes showed very little mitotic activity. Granulocytes of the mature type were very few. The reticulo-endothelial tissue showed here and there large round cells which were phagocytic. Large clumps of pigment and parasites were ingested. Numerous lymphoid cells were also present.

The Lung.

Histopathological changes in the lung were studied in three of these cases. All showed numerous para-:sites in the alveolar capillaries. Concentration was very marked in one case which showed an inter-:stitial pneumonia where it was difficult to decide if there was an overgrowth of the septal cells in addition to fibroblastic growth. Inflammatory changes with cell infiltration into the alveoli were not met with./

with. In one case the alveolar walls were beaded by the distension of the capillaries with parasites. Swelling of the capillary endothelium was the rule. Very few parasites were inside the alveoli and there was no sign of phagocytosis by the lining epithelium. In one case, the parasites appeared ingested by the septal cells. Small haemorrhages and larger infarc-;tions such as described in severe tertian malaria were not met with.

The Brain.

The brain was examined in three cases. All showed numerous parasites in the cerebral capillaries. Hyaline thrombi were not met with. In one case the parasites were so numerous as to suggest a cerebral localisation. There was also slight perivascular cuffing. Close to the cortex a large vessel was stuffed with parasites and immediately below was an area of softening. Perivascular haemorrhages were not met with. In no case was there any focal glial proliferation and granuloma formation such as described by Dürck (1925) in human malaria. The nerve cells showed chromatolysis in places.

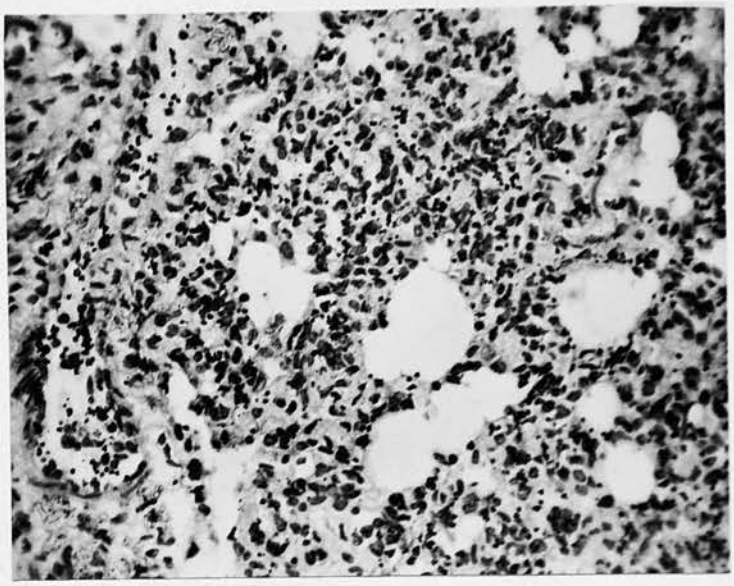

Fig.XIV.. Lung (x 350) showing chronic interstitial overgrowth in the alveolar walls. There is no ingestion by the flat lining epithelium. Note the parasitic chains in the alveolar capillaries. (Haemalum and Eosin).

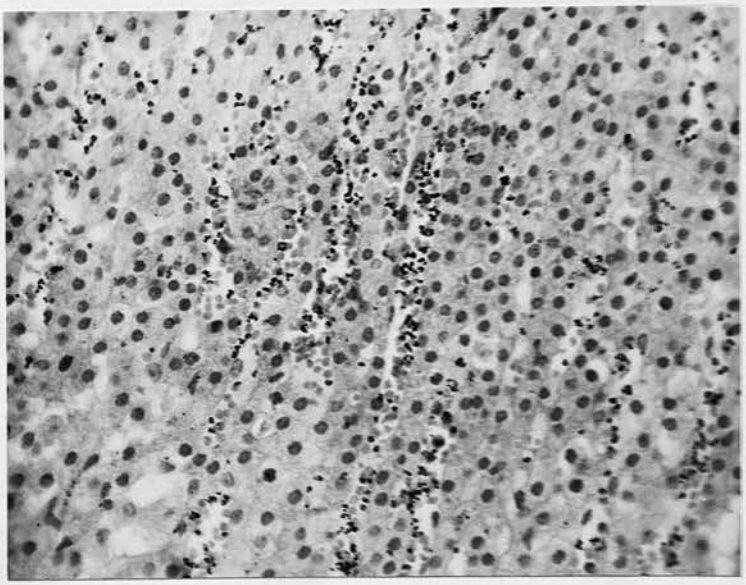

Fig.XV.. Adrenal K.7. (x 350) showing the capillaries of the zona fasciculata distended with parasite laden cells. (Haemalum and Eosin).

DISCUSSION.

The acute enlargement of the spleen with the distension of the capsule and the separation of the trabeculae may result either from mechanical distension from vascular engorgement or from cell infiltration, from degenerative swelling of the splenic pulp, from a paralysis of innervation of the capsulo-trabecular system, or from a direct toxic effect on the muscle and collagen bundles which would result in vascular stasis. A true hypertrophy of the splenic tissue would show itself in a more chronic type of progressive enlargement. In his perfusion experiments with warm saline Lubarsch (1927) found that mechanical distension caused an enlargement only up to twice the normal. The presence of oedema of the trabeculae, occasional trabecular softening with loss of staining of the nuclei of the muscle and collagen bundles, suggest a direct toxic effect, in addition to marked vascular engorgement in the present series of cases. The meshes of the pulp are so packed with red blood cells that the venous sinuses are compressed and often collapsed, so that an active hyperaemia might be held to occur. Obvious venous stasis would be shown by a distension of the venous sinuses and a later and a more gradual percolation into the pulp. In haemolytic jaundice, we find the existence of a mechanism which causes/

causes an active hyperaemia of the pulp leaving the sinuses free. It is possible that in malaria, a similar mechanism might exist whereby the parasite laden red corpuscles are brought into intimate relation with the phagocytic reticulum cells. Evidence of this selective localisation in the spleen, the liver and the bone marrow is brought forward by Taliaferro (1932) who found the greatest concentration of parasites in the spleen, next in the liver and then in the bone marrow, as compared with the concentration in the circulating blood. In the twelve cases now studied, parasitic localisation and vascular engorgement were much more marked in the spleen than in the liver, the bone marrow, the adrenal, the lung, the kidney or the heart muscle. The vascular engorgement of the spleen pulp cannot therefore be regarded as part of a general vascular reaction which has been argued to occur in malaria in man (Tirumurti and Rao, 1936) in the splanchnic circulation in antithetic reciprocal relation to the cutaneous pallor and vaso-constriction during the rigor. Adhesion of parasites and infected red corpuscles to the reticular mesh is so well marked in the spleen and the liver, as to suggest a general fixing effect on the parasite-laden red cells. A physiological vaso-dilatation would be a natural sequence. The recent work of Findlay and Brown (1934) seems to suggest/

suggest that the electrolyte behaviour of the parasite laden red corpuscles may be altered to account for this phenomenon.

Evidence for a hyperplastic lymphoid reaction is shown by: 1. the enlargement of the follicles and the activity of the germ centres; 2. the extension of the lymphoid sheaths of the arteries to the trabecular vessels; and 3. the subendothelial lymphoid reaction of the trabecular veins. Malamos (1934) in his study of the blood picture in acute monkey malaria refers to the relative increase in the lymphocytes in the blood apart from the mononuclear increase. This hyper-:plastic lymphoid reaction is of some significance. Lubarsch defines the splenic reaction in acute infec-:tions as of two differing types, the one affecting the pulp that is common in all septicaemias and allied conditions where organisms are actually present in the spleen and the other affecting the lymphoid follicles where there is a general toxaemia from a local infec-:tion. In diphtheria, there is thus a marked follicle reaction with enlargement and proliferation of the germ centres.

The occurrence of marginal annular necrosis and often of central necrosis is of great interest. Since Feitis (1921) drew attention to the presence of necrosis in the follicles due to the presence of small thrombi in a case of arterio sclerosis, many such cases have/

have been reported. Feitis, and Meuret (1925) associated the changes to arterio-sclerosis while Geipel and Matthias (1924) and Wilton (1925) described cases associated with acute infection. Enzer (1926) associated his case with obstruction in the sinus due to littoral proliferation while Spier (1931) held that the changes are not due to the formation of secondary thrombi but to an arteriolar necrosis. The presence of annular necrosis within the marginal zone is of some importance since according to Thoma (1924) the small penicillar branches of the central artery end in ampullar terminations at the marginal zones beyond which the sinuses commence. Degenerative changes in this zone suggest a toxic lesion on the capillaries. Recently Orr (1932) has described typical annular necrosis in the follicles in experimental streptococcal septicaemia in rats where the streptococcal localisa-:tion in the spleen was first noticed in the peri-:malpighian zone. In monkey malaria where the capillaries are filled with parasites, there is either a direct block or a toxic effect on the marginal capillaries. It is however difficult to implicate a blockage mechanism in the loose annular capillary mesh work. In the sinuses, this is still more improbable even though hyperplastic changes affect the littoral cells.

With regard to the development and extent of the phagocytic/

phagocytic mechanism in the spleen, Taliaferro and Carron (1936) have found, in Panaman monkeys infected with Plasmodium brasilianum that the phagocytic mechanism comes into action rather suddenly with a crisis when the parasites disappear from the blood. During this crisis numerous endoglobular parasites are ingested in toto by the phagocytic cells derived from the reticulo-endothelium . They regard the mechanism of immunisation as entirely due to the activated reticulo-endothelium which shows increased phagocytic activity. On the other hand, in this series of Plasmodium knowlesi infections in Indian monkeys, the phagocytic mechanism appears to be present throughout the infection. This is most active to free granules of pigment which are ingested with great avidity. Free merozoits, damaged parasites, parasitic débris, endoglobular parasites and damaged erythrocytes all appear to be equally ingested. There is no crisis with the sudden drop in the number of parasites in the blood, but the infection increases in intensity until the animal succumbs. Toxic lesions affecting the lymphoid cells and the lymphoid reticulum appear in the spleen, while the fixed reticulum cells and the littoral cells show marked differentiation into free amoeboid cells which show marked phagocytic activity. Active ingestion of parasites and pigment granules were present in all the specimens studied and this was

not/

not only by the free cells, but by the primitive cytoplasmic reticulum and by the lining cells of the sinuses. The lymphoid reticulum cells of the follicles showed in places differentiation and phagocytosis. Occasionally the large epithelium like cells of the germ centres showed active phagocytosis indicating that histiogenetically these cells are allied to the reticulo-endothelium (cf. Maximow, 1927). The histological picture was not that of a lack of reticulo-endothelial response, as has been suggested by workers on immunity, but that of an overwhelming infection that developed in spite of a reticulo-endothelial reaction. The phago-:cytic reaction was equally well marked in the sinuses and showed itself in littoral differentiation into large mononuclear cells which ingested parasites and pigment granules. Further evidence of the activation of the reticulo-endothelium was shown by the extension of the sinus reaction along the pulp veins into the trabecular veins, where macrophage differentiation and phagocytosis gave rise to a picture of hyperplastic endophlebitis.

The possibility of vascular occlusion by parasitic plugs together with the finding of numerous parasites filling up the capillaries in different organs have lead to a mechanistic view of the pathogenesis of the lesions in malaria. In the present series mostly of hyper-acute/

hyper-acute infections in monkeys the concentration of parasites in the blood was so marked that localisa-:tion in many organs would be a natural sequence. The appearance of masses of parasites distending the vessels in different organs such as the adrenal, the lung, the brain, and the kidney in monkey malaria indicate the importance of such local concentrations in the pathology of the lesions in malaria. It is possible that a similar explanation might hold in human malaria where lesions have been described in the liver (Kelsch and Keiner, 1878; Barker, 1895; Duprey, 1907; Millons, 1924; Koltz, 1929; Hughes and Srivatsava, 1931), in the kidney (Moore, 1902; Marchiafava and Bignami, 1891; Clark, 1912; Swan, 1907; Bowman, 1910; Erdelyer and Kürz, 1917; Goldie, 1930; Giglioli, 1930; Surbek, 1931), in the heart muscle (Gaskell and Miller, 1920; McFie and Ingram, 1920; Mitchelletti, 1929), in the adrenal (Valenti, 1907; Elmassiau, 1911; De Brun, 1910; Paisseau and Lemaire, 1916; Dudgeon and Clark, 1919), and in the brain (Dürck, 1917; 1925; Seyfarth, 1927; van der Horst and Verharrt, 1934).

As regards the mechanism of such localisations it has been held that the spleen, the liver and the bone marrow are organs of predilection of the parasites where schizogony can take place. On the other hand, the filtering effect of the reticulo-endothelial mesh would/

would seem to be responsible for such selective con-:centrations. The localisation and lesions in other organs in hyperacute infections would seem to depend on a fortuitous delay or arrest in capillary vessels followed by rapid local multiplication with consequent blockage.

SUMMARY.

A histopathological study of twelve cases of fatal monkey malaria due to infection with P. knowlesi has shown: (1) the presence of a vascular mechanism that localises the parasites in large numbers in the spleen; (2) the presence of a lymphoid reaction in the spleen; and (3) the activation of the reticulo-endothelial system as shown by histiocytic differentiation and active phagocytosis.

The severity of the infection appears to be due, not to a failure of the reticulo-endothelial response, but to some other factor which may be the virulence of the parasites for a particular host species.

Degenerative swelling of the capillary endothelium with necrosis of the cytoplasmic reticulum and the follicles suggest a toxic factor; some of the other lesions met with can be explained by the theory of mechanical localisation of parasites in the capillaries of the affected part.

The/

The lesions met with in monkeys in other organs besides the spleen, suggest that such localisations may be responsible for the varied changes in different organs in human malaria also.

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PATHOLOGICAL STUDIES ON SPLENOMEGALY.

PART II. SPLENOMEGALY IN MAN.

CHAPTER I.

THE SPLENOMEGALY OF HEPATIC CIRRHOSIS AND LIVER NECROSIS AND ITS RELATION TO SPLENIC ANAEMIA.

THE SPLENOMEGALY OF HEPATIC CIRRHOSIS AND LIVER NECROSIS AND ITS RELATION TO SPLENIC ANAEMIA.

INTRODUCTION.

The association of splenomegaly with cachexias, miasmatic states, and even haemorrhages, finds mention in the writings of Hippocrates. In modern medicine, with Virchow's recognition of the leukaemias as a separate entity, cases of splenomegaly with anaemia and lymphatic enlargement, but without leukaemic changes in the blood, came to be grouped together, though still under various names as Hodgkin's disease (Wilks), pseudo-leukaemia (Cohnheim), lymphosarcoma (Virchow), and malignant lymphoma (Billroth). Yet another group remained where the anaemia and splenomegaly were not associated with lymphatic enlargement. Gretsel (1866) in Griesenger's clinic described such a case under the term "anaemia splenica" as distinct from "anaemia lymphatica" which had been applied for Hodgkin's disease. In German literature we find the label "anaemia splenica" applied to various clinical conditions. Thus, under this term, Strumpell (1876, 1877) described cases more or less resembling pernicious anaemia, while

Senator/

Senator (1905) and Micheli (1906) confused the issue with cases described as pseudoleukaemia. Other names were also applied to this condition as for instance, primitive splenomegaly (Debove and Brühl, 1892), idiopathic hypertrophy of the spleen without leucocythaemia (Gaucher, 1892). In Italian litera-:ture Banti (1883) gave a full account of the disease and its clinical features of secondary anaemia, leucopenia and splenomegaly. Banti also drew atten-:tion to a histological change in the malpighian follicles, a "sclerose del folliculo" which he regarded as the essential lesion. Most German haematologists led by Naegeli (1905) and Sternberg (1905) criticised this conception of a splenic anaemia which they regarded as only a syndrome and not a morbid entity. Splenomegaly with anaemia was the predominant feature in such varying clinical conditions as tuberculosis, malaria, tumours and precirrhotic states, so that there was little justification for the term "splenic anaemia". In spite of this criticism however, in English and American literature the term "splenic anaemia" has survived mostly through the writings of Wood (1871) of Osler (1899, 1900, 1902) of Sippy (1899) of Jackson (1901) of Stengel (1904) and many other clinicians. Thus Osler (1902) care-:fully analysed his cases and redefined the disease "as/

"as a chronic affection probably an intoxication of unknown origin which causes a progressive splenomegaly, a marked tendency to haemorrhage from the stomach, and in most cases a terminal stage of liver cirrhosis, jaundice and ascites". He drew attention to its duration, to the splenomegaly which preceded the anaemia and to the absence of hepatic damage in the earlier stages, features which Banti had also emphasised in his pioneer studies. This view of a primary splenomegaly (Banti, 1833) which might even be the cause of the anaemia (Sippy, 1898), directed attention to the spleen as the offending organ; and the operative treatment by splenectomy came into vogue and is favoured even today.

The frequency of splenic enlargement in portal cirrhosis is also well established. The more reliable figures range from 80% in Kolpstock's series (Kolpstock, 1907), 83% in Naunyn's series (Naunyn, 1904) to 93% in Bamberger's series (Bamberger, 1907). The enlargement is generally moderate averaging 12.9 ozs. (353 gm.) in Kelynack's series (Kelynack, 1897) and 400 gm. in Lubarsch's series (Lubarsch, 1928). In biliary cirrhosis of the Hanot type splenomegaly is even more marked so that Chauffard (1900) distinguished various clinical types, splenomegalic, hyperpplenomegalic and meta-splenomegalic, in the last/

last the splenic enlargement preceding the liver cirrhosis. In the toxic type of Mallory (1911) splenic enlargement is also quite frequent.

Rolleston and McNee (1929) distinguished types one with well established and fatal cirrhosis, another where splenomegaly is part of a latent or healed cirrhosis where portal decompensation was not in evidence. In Banti's syndrome the sequence of events is a primary splenic anaemia followed by a late cirrhosis. Bastai's familial splenomegaly, (Bastai, 1922) the splenomegaly of Egypt, ~~xxx~~ De's Bengal splenomegaly (De, 1932), ~~xxxxx~~ are probably similar types. Eppinger's conception of spleno-hepatic correlation tends to group together all the differing types and McMichael (1934) uses the term "hepatolienal fibrosis" as he inclines to stress the liver damage in these groups.

The splenic enlargement was first attributed to passive congestion from increased venous pressure in the portal circulation brought about by the obstruction of the portal capillary bed in the liver. Senator (1893) however suggested an additional factor in a direct damage to the spleen by the same agent that caused the liver cirrhosis. Seiveking (1894) who investigated the changes found no proliferation of connective tissue in the pulp corresponding to the liver/

liver cirrhosis except a capsulo-trabecular thickening. He held that the changes were due to simple congestion. On the other hand Oesterich (1895), on the basis of histological studies described a "hyperplasia pulpae lienis" in cases where he held that secondary infec-:tions could not account for the splenic reaction. He concluded that splenic hyperplasia and cellular infiltration were phases in early stages of cirrhosis. Bleichroeder (1904) however argued that the splenic tumour was primary and pre-cirrhotic and that histo-:logically a lymphocytosis of the sinuses and the veins was the predominant picture, suggesting a primary blood condition. Chauffard's view of a splenomegalic cirrhosis is the expression of a liver damage due to poisons manufactured in the spleen. Further histo-:logical studies by Kolpstock (1907) served to separate the cirrhotic spleens from those produced by venous congestion as in cardiac failure. He described the forms in incipient cirrhosis as similar to the splenic tumour in infections, the enlargement beginning even before congestion had developed, and being softer in consistence, and of a paler red colour than the purplish blue, indurated, and smaller spleens of cardiac stasis. A direct toxic damage of the spleen was a more probable cause to account for the enlarge-:ment. The idea of a precirrhotic splenomegaly, as distinct/

distinct from a simultaneous toxic effect, had however found very many adherents among German workers (Theirfelder, 1898; Leichtenstern, 1896; Hermann, 1901). In French literature, we find Gauckler (1904) distin-:guished two types of cirrhotic spleen, a small "sclerose atrophique" type with increase of connective tissue spreading from the vessels and trabeculae and a large "sclerose hypertrophique pulpaire" where the splenomegaly was due to an increased erythrolysis in the spleen. Still another view is that of Hartwich (1912), who following Grawitz's teaching, suggested that the splenomegaly was a compensatory process to make up for the liver damage. Later Strasser (1922) supported this view. In his extensive studies Eppinger (1920) found that the spleens in hypertrophic liver cirrhosis were histologically very similar to those of Banti's disease. He concluded that the splenomegaly was not proportional to the extent of the liver cirrhosis and that "the spleen possessed in liver cirrhosis autonomy up to a certain degree". "It may get diseased with the liver, but is not equally severely affected in every case". McNee (1932) in his Croonian lectures has advanced the theory that with portal obstruction there is an alteration in the hepatic circulation due to dissociation of hepatic structure by cirrhosis, and that the portal blood is diverted/

diverted through collateral channels and enters the general circulation; the metabolic products in the portal blood stream are not detoxicated by the liver but go "past the liver into the general circulation and reach the liver and spleen simultaneously in the arterial blood!"; the splenomegaly is the result of this alimentary toxaemia together with the congestive factor from portal stasis.

MATERIAL AND METHODS OF STUDY.

Material for study consisted of histological sections of spleens removed at autopsy at the Patho-:logical Department of the Royal Infirmary, Edinburgh, and from specimens of splenectomy from the Department of Surgery, Edinburgh University. On the whole, specimens were obtained from autopsy material in 52 cases of liver disease. Of these 38 cases were of portal cirrhosis, 7 of acute or subacute liver atrophy, one of acholuric jaundice with cirrhosis, one of cirrhosis with haemochromatosis, one of syphilitic cirrhosis (hepar lobatum), one of focal necrosis of the liver with splenomegaly, 2 of primary carcinoma of the liver with cirrhosis and one of primary carcinoma of the gall bladder with secondary deposits in the liver. Out of 63 specimens from the Surgery Department a careful study showed that 25 could be grouped together as belonging to the splenic anaemia group. Of the rest, 22 were of acholuric jaundice, 9 of essential thrombocytopenia, 4 of myeloid meta-:plasia, 1 of lymphoid reticulosis, 1 of pernicious anaemia and 1 of tumour deposit. Four specimens of a type of splenic anaemia met with in Bengal, called Bengal splenomegaly, were obtained from Professor M.N. De of the Bengal Medical College.

In most of these cases where paraffin blocks were/

were available sections were stained by (1) Mayer's haemalum and eosin, (2) Heidenhain's azan stain, (3) Leishman's stain for tissues as described in the appendix, (4) Wilder's modification of the Foot-Bielschowsky method for the demonstration of reticulum (Wilder, 1935), (5) Perl's prussian blue reaction. In a few cases frozen sections were stained for the demonstration of fat and lipoid material. Verheoff's elastic tissue stain was also occasionally used to study the changes in the elastic tissue. Where fresh material was available Turnbull's blue reaction by pre-treatment with ammonium sulphide was carried out in order to compare the result's with the Perl's reaction. Where the material was fixed in Zenker, Perl's reaction was carried out without removing the mercury crystals by treating with iodine and hypo-:sulphate, so that any iron salts present may not be dissolved out by the iodine. Most of the splenectomy material was fixed in Jöres fluid while the autopsy material was fixed in Helly's fluid. In recent cases blocks were obtained from different places of the same spleen. In 18 cases of the splenectomy series the splenic vein was examined histologically.

TABLE I.

Spleens removed at autopsy.

Serial No.	Specimen No.	Name	Age	Clinical Abstract	Anatomical Diagnosis	Inflammatory Complication	Histology								Inflammatory infiltration	Remarks Type of Reaction
							Capsular thickening	Trabecular thickening & splitting	Siderotic nodules	Peri-arter-:ial fibro-:sis & other lesions	Malpighian follicles	R.E. Proli-:feration	Sinus Pro-:liferation	Congestion = C Haemorrhage = H		
1.	D.B.703	L.S.	10	Splenic anaemia type Banti; liver enlarged; Haematemesis; lymphadenitis.	Subacute liver atrophy; toxic visceral changes; splenomegaly.	Bronchitis	-	-	+	+	Germ centres	+ Necro-:sis	+	-	Lymphocytic Leucocytic Monocytic	Haemosiderosis; Focal necrosis. Type: Proliferative and inflammatory.
2.	D.B.1337	B.S.	59	Heart trouble	Cirrhosis hepatis; fatty heart; venous engorgement.	Bronchitis	+	+	+	+	No change	-	-	Sinus-(C) Pulp-(C)	-	Type: Congestive.
3.	D.B.1806	J.B.	64	Ascites; haematemesis.	Cirrhosis hepatis; emphy-:sema & oedema lung; contracted kidneys; en-:larged fibrotic spleen.	Hypostatic pneumonia	++	++	-	+	Atrophy	+	++	Sinus-(C)	-	Fibro-adenie + Type: Proliferative and fibrotic.
4.	D.B.1871	Mrs. I.C.	63	Pylorectomy for carcinoma.	Cirrhosis hepatis; no portal obst-:ruction.	Nil	-	-	-	+	Atrophy	+ Necro-:sis	-	-	Lymphocytic	Type: Proliferative and necrotic.
5.	D.B.1906	J.T.	59	Ascites; jaundice; nausea.	Cirrhosis liver; contracted kid-:neys; hypertrophy stomach and intestinal wall.	Acute strepto-:coccal peritonitis	+	+	+	Hyaline early fibril-:ary in-:crease cuffing	Atrophy	-	+	Periarter-:ial-(H) Pulp-(C)	Monocytic Plasmacytic	Type: Congestive and inflammatory.
6.	D.B.1932	Mrs. A.H.	59	Oedema legs & abdomen; ascites; jaundice.	Subacute liver atrophy; gall stones; adherent pericardium; fatty heart, splenomegaly.	Nil	-	?	-	+	Atrophy	-	?	Sinus-(C) Pulp-(H)	Lymphocytic	Type: Congestive.
7.	D.B.1939	Mrs. J.C.H.	28	Ascites; splenomegaly; anaemia.	Cirrhosis liver; ascites; enlarged oesophageal veins; spleen - 1160 gm.	Nil	++	+	-	+	Atrophy	+	+	Sinus-(C) Pulp-(C)	Monocytic	Fibro-adenie ++ Type: Proliferative and fibrotic with congestion.
8.	D.B.1973	Mrs. A.C.	56	Nausea; en-:larged liver; ascites.	Primary carcinoma with cirrhosis; liver; spleen 550 gm.	Nil	+	++	+	+	Atrophy	+	+	Sinus-(C)	Lymphocytic Plasmacytic	Sub-capsula fibro-adenie. Type: Proliferative and fibrotic.

TABLE I (continued).

Serial No.	Specimen No.	Name	Age	Clinical Abstract	Anatomical Diagnosis	Inflammatory Complication	Histology								Inflammatory infiltration	Remarks Type of Reaction
							Capsular thickening	Trabecular thickening & splitting	Siderotic nodules	Peri-arterial fibrosis & other lesions	Malpighian follicles	R.E. Proliferation	Sinus Proliferation	Congestion = C Haemorrhage = H		
9.	D.B. 2033	J.N.	46	Ascites; jaundice splenomegaly abdomen tapped 8 times.	Cirrhosis liver; perihepatitis; spleen 880 gm. jaundice.	Nil	+	+	-	++	Atrophy	-	+	Sinus-(C) Pulp-(C)	-	Fibro-adenie + Type: Congestive and fibrotic.
10.	D.B. 2075	Mrs. M.M.	65	Abdominal pain; haematemesis.	Cirrhosis liver; old pleurisy; splenomegaly; peri-splenitis.	Nil	+	+	-	+	Atrophy	-	-	-	Lymphocytic Leucocytic Plasmacytic	Fibro-adenie + Type: Inflammatory and fibrotic.
11.	D.B. 2255	Mrs. M.	66	Haematemesis.	Cirrhosis liver; haemorrhage from oesophageal varix; spleen 568 gm.	Nil	+	++	-	+	Atrophy lympho-rehxis	+	?+	Sinus-(C)	Monocytic Plasmacytic	Early fibro-adenie. Type: Inflammatory and fibrotic.
12.	D.B. 2338	A.C.	59	Operated for necrosis ankle; developed ascitis, slight jaundice.	Cirrhosis liver; chronic duodenal ulcer; congestion; oedema lungs; spleen 295 gm.	Nil	?	+	-	-	Atrophy	+	-	Sinus-(C) Pulp-(C)	Leucocytic Lymphocytic Monocytic	Type: Inflammatory and congestive.
13.	D.B. 2370	A.L.	53	Ascitis; jaundice; oedema legs.	Cirrhosis liver; congestion, oedema lungs; spleen 220 gm.	Patch of pneumonia right lung	-	+	-	+ Hyaline change	Atrophy	+	?+	Pulp-(C)	Monocytic Plasmacytic Lymphocytic	Type: Proliferative and inflammatory.
14.	D.B. 2372	A.B.	58	Cough; breathlessness; haematemesis.	Cirrhosis liver; fibrosis lung tuberculous ? spleen twice normal size.	Nil	+++	++	-	+	Atrophy	-	-	Sinus-(C) Pulp-(C)	-	Type: Congestive and fibrotic. Haemosiderosis.
15.	D.B. 2411	A.R.	39	Vomiting; jaundice; enlarged liver; enlarged glands.	Primary cancer gall-bladder; liver; gall-stones tuberculosis; splenomegaly.	Malignant peritonitis	-	-	-	+	Germ centres	+	-	-	Lymphocytic Monocytic	Type: Subacute inflammatory.
16.	D.B. 2453	I.A.	71	Jaundice; loss of weight; nausea.	Acute yellow atrophy; toxic visceral changes; spleen 165 gm.	Nil	-	-	-	+ Hyaline necrosis	Active	- Necrosis	-	Sinus-(C) Pulp-(H) Diffuse-(H)	Leucocytic Lymphocytic	Type: Congestive. Haemorrhagic necrosis.

TABLE I (continued).

Serial No.	Specimen No.	Name	Age	Clinical Abstract	Anatomical Diagnosis	Inflammatory Complication.	Histology								Inflammatory infiltration	Remarks Type of Reaction
							Capsular thickening	Trabecular thickening & splitting	Siderotic nodules	Peri-arter-:ial fibro-:sis & other lesions	Malpighian follicles	R.E. Proli-:feration	Sinus Pro-:liferation	Congestion = C Haemorrhage = H		
17.	D.B.2490	A.H.	59	Oedema legs; ascitis; slight jaundice; veins enlarged.	Cirrhosis liver; fibrosis myocar-:dium; venous congestion; haemorrhage stomach.	Nil	+	++	+	++	Atrophy	-	-	Pulp-(C) Pulp-(H)	Lymphocytic	Type: Congestive.
18.	D.B.2585	Specimen of Dr. Davidson's from a case of Banti's disease.					+	+ infilt-:ration	-	+ cuffing	Atrophy	++	?+	Sinus-(C) Pulp-(C)	Monocytic	Fibro-adenie +++ Type: Proliferative with congestion.
19.	D.B.2594	W.J.	49	Cough; haemop-:tysis; enlarged spleen	Syphilis of dura, skull, aorta, lungs hepar lobatum; spleen 420 gm.	Nil	+	++	-	+ cuffing	Atrophy	+	?+	Sinus-(C)	Monocytic Plasmacytic	Fibro-adenie Type: Proliferative.
20.	D.B.2695	F.W.	39	Oedema legs; ascitis; jaundice.	Cirrhosis liver; cloudy swelling kidney, myocardium; spleen 400 gm.	Nil	+	++	+	+	Atrophy	?	+	Sinus-(C) Subcapsular & Follicular-(H)	Monocytic	Fibro-adenie ++ Type: Congestive and fibrotic.
21.	D.B.2709	A.H.	49	Oedema legs; ascitis; rt. pleural effusion; haematemesis.	Cirrhosis liver; ascites; enlarged spleen.	Pleurisy right side	+	+	-	+	Atrophy	-	+	Sinus-(C) Pulp-(H)	Lymphocytic	Type: Congestive.
22.	D.B.2710	Mrs. M.S.	52	Jaundice; oedema face; ascitis; rt. pleural effusion; haematemesis.	Acute yellow atrophy; ascitis; adenoma thyroid; spleen 280 gm.	Nil	+	++	+	+ cuffing	Atrophy	-	-	Sinus-(C) Pulp-(C)	Leucocytic	Type: Congestive and inflammatory.
23.	D.B.2757	J.S.	55	Ascitis; jaundice; oedema legs.	Primary cancer, with cirrhosis liver; portal obstruction; spleen twice normal size.	Nil	+	++	+	+	Atrophy	+	-	Trabeculae-(H) Sinus-(C)	Leucocytic Plasmacytic	Subcapsular fibro-adenie Type: Congestive and fibrotic.
24.	D.B.2890	E.B.	51	Jaundice; ascitis; double aortic murmer.	Cirrhosis liver; thickening aortic cusps; ascitis; spleen 680 gm.	Nil	-	+	+	+	Atrophy	+	+	Sinus-(C) Pulp-(C) Subcapsular-(H)	-	Fibro-adenie ++ Type: Congestive and fibrotic.

TABLE I (continued).

Serial No.	Specimen No.	Name	Age	Clinical Abstract	Anatomical Diagnosis	Inflammatory Complication	Histology								Inflammatory infiltration	Remarks Type of Reaction
							Capsular thickening	Trabecular thickening & splitting	Siderotic nodules	Peri-arterial fibrosis & other lesions	Malpighian follicles	R.E. Proliferation	Sinus Proliferation	Congestion = C Haemorrhage = H		
25.	D.B. 2913	W.W.	68	Lead poisoning previously; oedema legs; cough.	Cirrhosis liver; ascitis; spleen 3 times normal size.	Nil	++	++	-	++	Atrophy	+	+	Sinus-(C) Pulp-(C)	Monocytic Leucocytic	Type: Inflammatory and fibrotic.
26.	D.B. 3106	Mrs. H.D.	30	Haematemesis; coma.	Cirrhosis liver; haemochromatosis; spleen twice normal size.	Nil	+++ 1500	+++	+	+	Atrophy	-	-	Pulp-(C)	Lymphocytic	Type: Congestive with capsulo-trabecular fibrosis.
27.	D.B. 3136	C.B.	40	Haematemesis; epistaxis; stupor.	Cirrhosis liver; ascitis; oesophageal varix rupture.	Nil	++	+++	+	+	Atrophy marked	-	-	Trabecular-(H) Sinus-(C) Pulp-(C)	Lymphocytic	Type: Congestive with capsulo-trabecular fibrosis.
28.	D.B. 3147	Mrs. A.S.	45	Ascitis; menorrhagia; fever.	Cirrhosis liver; ascitis; aortic incompetence; spleen twice normal size.	Nil	- Cell infiltration	Focal Necrosis + cuffing of veins	-	+ cuffing	Active Lymphorehxis	-	-	Sinus-(C) Pulp-(C)	Leucocytic Lymphocytic	Type: Congestive, inflammatory and necrotic.
29.	D.B. 3152	F.G.	61	Nausea; vomiting; epistaxis; ascitis.	Cirrhosis liver; dilated heart; spleen 6 times normal size.	Nil	+	+	-	+	No change	+	-	Sinus-(C)	Monocytic Lymphocytic	Type: Inflammatory.
30.	D.B. 3256	Mrs. A.	54	Gangrenous appendix removed.	Cirrhosis liver; oesophageal varix; enlarged spleen.	Purulent bronchitis	-	+	-	+	Atrophy	+	-	Sinus-(C) Pulp-(C)	Monocytic Lymphocytic Plasmacytic	Type: Congestive and inflammatory.
31.	D.B. 3362	J.F.	46	Arsenical treatment for syphilis; jaundice; coma.	Subacute liver atrophy; ascitis; spleen 670 gm.	Bronchopneumonia	+	+	-	+	Slight atrophy	-	-	Follicle-(H) Pulp-(H) Sinus-(C) Pulp-(C)	Monocytic	Type: Congestive.
32.	D.B. 3489	J.D.	64	Cholecystotomy for obstructive jaundice; cirrhosis liver; chronic pancreatitis.	Cirrhosis liver; ascitis; spleen enlarged.	Nil	+	++	-	+ Hyaline	Atrophy	+	+++	Follicle-(H)	-	Fibro-adenie ++ Type: Proliferative and fibrotic.

TABLE I (continued).

Serial No.	Specimen No.	Name	Age	Clinical Abstract	Anatomical Diagnosis	Inflammatory Complication	Histology								Inflammatory infiltration	Remarks Type of Reaction
							Capsular thickening	Trabecular thickening & splitting	Siderotic nodules	Peri-arterial fibrosis & other lesions	Malpighian follicles	R.E. Proliferation	Sinus Proliferation	Congestion = C Haemorrhage = H		
33.	D.B. 3542	D.M.	68	Sleepiness; tremor; nystagmus; Romberism.	Cerebellar tumour; cirrhosis liver; spleen 800 gm.	Hypostatic pneumonia	+	+	-	+	Active	+	-	Sinus-(C)	Monocytic	Type: Early proliferative.
34.	D.B. 3545	A.M.	61	Bronchitis; haemoptysis.	Cirrhosis liver; rupture oesophageal varix; chronic pancreatitis; spleen 250 gm.	Early broncho-pneumonia	+	+	-	+	Slight atrophy	+	-	Sinus-(C)	Lymphocytic plasmacytic monocytic	Type: Early proliferative and inflammatory.
35.	D.B. 3546	A.M.	33	Jaundice; anaemia; haematemesis; fragility increased.	Acholuric jaundice; early liver cirrhosis; oseophageal varix; spleen 1890 gm.	Nil	+	+	+++	+	Marked atrophy	++	?	-	-	Pulp fibrosis. Type: Fibrotic.
36.	D.B. 3565	R.M.	37	Anti-syphilitic treatment; signs of perforated ulcer duodenum.	Cirrhosis liver; chronic ulcer duodenum with haemorrhage; spleen 115 gm.	Nil	-	+	-	+	Active germ centres	-	-	Sinus-(C) Pulp-(C) Peri-follicular-(H)	Polynuclear Lymphocytic	Type: Congestive and inflammatory.
37.	D.B. 3659	M.S.	56	Jaundice; nausea; pain abdomen.	Acute yellow atrophy mitral stenosis dilated heart; cloudy swelling viscera; enlarged spleen.	Nil	-	+	-	+	Active	-	-	Sinus-(C) Pulp-(C) Peri-malpighian-(H)	Leucocytic Lymphocytic	Type: Congestive and inflammatory.
38.	D.B. 3715	Mrs. L.		Haematemesis; operated for gall-stones.	Cirrhosis liver; oesophageal varix; spleen enlarged.	Nil	-	+	-	+	Atrophy	+	+	-	Lymphocytic Monocytic	Fibrillary increase + Type: Proliferative and fibrotic.
39.	D.B. 3758	Mrs. A.B.	53	Oedema legs; ascitis; breathlessness.	Cirrhosis liver cholilithiasis; obstruction cystic duct; spleen twice normal size.	Nil	-	+	-	+	Atrophy	+	-	Sinus-(C)	Lymphocytic	Fibrillary increase + Type: Congestive and fibrotic.
40.	D.B. 3904	W.W.	51	Attacks of jaundice.	Cirrhosis liver; oesophageal varix; subacute endocarditis; spleen 8 times normal size.	Nil	-	+	-	+ Necrosis	Germ centres necrosis	-	-	Peri-arterial-(H) Sinus-(C) Pulp-(C)	Leucocytic Lymphocytic Plasmacytic	Type: Congestive and inflammatory.

TABLE I (continued).

Serial No.	Specimen No.	Name	Age	Clinical Abstract	Anatomical Diagnosis	Inflammatory Complication	Histology									
							Capsular thickening	Trabecular thickening & splitting	Siderotic nodules	Peri-arter-:ial fibro-:sis & other lesions	Malpighian follicles	R.E. Proli-:feration	Sinus Pro-:liferation	Congestion = C Haemorrhage = H	Inflammatory infiltration	Remarks Type of Reaction
41.	D.B.4127	Mrs. L.	Specimen of Dr. Arnott's.				+	+	-	++ cuffing	Atrophy necro-:sis	-	-	Pulp-(C) & (H)	Leucocytic Lymphocytic	Type: Congestive and inflammatory.
42.	D.B.4128	L.S.	18	Coma; cerebral irritation.	Cirrhosis liver; enlarged spleen; soft pale heart muscle; salpingitis.	General peritonitis	-	+	-	+ cuffing	Active lympho	+	+	Trabecular-(H) Follicle-(H) Sinus-(C)	Monocytic Lymphocytic	Type: Early proliferative and congestive.
43.	D.B.4383	T.T.	76	Confluent broncho-pneumonia.	Cirrhosis liver; oesophageal varix aplastic marrow.	Lobar pneumonia	-	+	-	+	Atrophy	+	+	Sinus-(C) Pulp-(C)	Monocytic Polynuclear Lymphocytic Plasmacytic	Type: Inflammatory and proliferative.
44.	D.B.4477	J.L.	50	Weakness; loss of weight; haematemesis.	Cirrhosis liver; oesophageal varix with rupture; spleen 5 times normal.	Nil	+	+	-	++	Atrophy	-	-	Follicle-(H) Pulp-(C) & (H)	Leucocytic Plasmacytic Lymphocytic	Fibrillary increase +++ Type: Congestive and fibrotic.
45.	D.B.4515	J.A.	68	Ascitis; loss of weight; tumour abdomen; oedema legs.	Ulcer cancer stomach; cirrhosis liver; ascitis; malignant peritonitis.	Nil	-	+	-	+	Atrophy	+	+	-	-	Fibrillary increase ++ Type: Proliferative and fibrotic.
46.	D.B.4529	G.M.	67	Ascitis; abdominal pain; haematemesis.	Cirrhosis liver; oesophageal varix carcinoma pan-:creas; deposit liver, glands.	Nil	++	++	-	+	Atrophy	++	++	-	Monocytic	Fibrillary increase + Type: Proliferative.
47.	D.B.4565	H.P.	34	Nausea vomiting; haematemesis; jaundice.	Cirrhosis liver; varicose oesopha-:geal veins; enlarged spleen; ascitis; hydro-:thorax; chronic endocarditis.	Nil	++	++	+	++	-	++	+	Slight (C)	Monocytic	Subcapsular. Fibrillary increase. Type: Proliferative.
48.	D.B.4643	T.R.	29	History of syphilis; anasarca.	Cirrhosis liver; pleural, periton-:eal, pericardial effusions; spleen twice normal size	Pneumococcal peritonitis	+	+	-	+	Active	+	-	Follicle-(C) Sinus-(C)	Lymphocytic Leucocytic	Type: Proliferative and inflammatory.

TABLE I (continued).

Serial No.	Specimen No.	Name	Age	Clinical Abstract	Anatomical Diagnosis	Inflammatory Complication	Histology								Inflammatory infiltration	Remarks Type of Reaction
							Capsular thickening	Trabecular thickening & splitting	Siderotic nodules	Peri-arter-:ial fibro-:sis & other lesions	Malpighian follicles	R.E. Proli-:feration	Sinus Pro-:liferation	Congestion = C Haemorrhage = H		
49.	D.B.4645	M.R.	12	Haemoptysis; pain and tenderness in left hip.	Subacute liver atrophy; septic arthritis left hip; ilio-psoas abscess; spleen twice normal size.	Broncho-pneumonia	-	+	-	+ cuffing endothe-:lial swelling	Atrophy	+	-	Follicle-(C) & (H) Sinus-(C)	Lymphocytic Leucocytic	Type: Proliferative and inflammatory.
50.	D.B.4699	R.S.	26	Infantilism; ascitis; splenomegaly of Banti's type.	Bronchiectasis; dwarfism; fetal thyroid; focal necrosis liver; spleen 1100 gm.; cystic kidney.		+	+	+	+	Atrophy Hyaline	++	?+	Sinus-(C)	-	Fibrillary increase +++ Type: Proliferative and fibrotic.
51.	M.H.A. 104	G.S.	67	Alcoholism; fibrosis left lung; coma.	Cirrhosis liver; ascitis; oesopha-:geal varix; dilated heart; bronchiectasis.	Nil	++	+	-	+	-	++	++	Sinus-(C)	-	Fibrillary increase ++ Type: Proliferative and fibrotic.

TABLE II.
Splenectomy Group.

Serial No.	Specimen No.	Name	Age	Clinical Abstract	Size or weight of spleen	Histology: Capsular thickening	Trabecular thickening & splitting	Siderotic nodules	Peri-arterial fibrosis & other lesions	Malpighian follicles	R.E. Proliferation	Sinus Proliferation	Congestion = C Haemorrhage = H	Inflammatory infiltration	Remarks Type of Reaction
52	3842	R.S.	33	Antispecific treatment 5 years ago; jaundice; rigors.	1010 gm.	Slight	++	-	-	Atrophy	++	++	-	-	Pulp "Fibro-adenie" +++ Type: Proliferative and fibrotic
53	4637	Mrs. M.B.	49	Haematemesis; anaemia; splenomegaly.	Enlargement marked	Slight	++ Inflammation	++	+	Atrophy	+	++	-	-	Pulp "Fibro-adenie"++ Type: Proliferative
54	4938	G.G.	18	Splenomegaly; anaemia.	356 gm.	+	+ Cell Infiltration	-	- Focal infiltration	Germ-centres	++	+	-	-	Sub-capsular "Fibro-adenie" Type: Proliferative
55	4940	T.N.	47	Splenomegaly; anaemia.	521 gm.	+	+	+	+ Necrosis	Atrophy	+	+	-	Lymphocytic Monocytic	"Fibro-adenie" + Type: Early proliferative
56	5094	Mrs. M.		4 year's anaemia; splenomegaly.	enlarged.	-	-	+	Endothelial reaction	Atrophy a few germ centres	+++	+	Subcapsular (H)	-	Type: Proliferative
57	5254	H.C.	51	Anaemia; splenomegaly.	enlarged.	-	Slight	+		Active	+	?	Slight (C) Trabecular (H)	Leucocytic Lymphocytic	Sub-capsular "Fibro-adenie" - Mixed reaction
58	5259	Miss C.	25	Haematemesis; purpura; anaemia; splenomegaly.	enlarged.	+ Necrosis	++	+	++ Necrosis	"Fibro-adenie"	++	++	Subcapsular (H)	Slight -	Sub-capsular "Fibro-adenie" ++ Type: Proliferative
59	5334	D.L.	24	Weakness; anaemia; haematemesis; history of syphilis.	370 gm.	-	-	-	Endothelial reaction cuffing	No change	+++	?	Slight (C)	- Lymphocytic	Sub-capsular "Fibro-adenie" - Type: Proliferative
60	5355	P.		Splenomegaly; sec. anaemia; leucopenia; haemorrhoids.	501 gm.	-	+	-	-	Germ centres	++	+	-	Leucocytic Lymphocytic Monocytic	Type: Proliferative and inflammatory
61	5543	H.D.	54	Chronic splenomegaly; anaemia; jaundice; early cirrhosis.	enlarged.	+	+	-	Endothelial reaction	Atrophy	-	-	(C) +++	Lymphocytic Plasmacytic	Type: Congestive and inflammatory

TABLE II (Continued)

Serial No.	Specimen No.	Name	Age	Clinical Abstract	Size or weight of spleen	Histology									
						Capsular thickening	Trabecular thickening & splitting	Siderotic nodules	Peri-arter-:ial fibro-:sis & other lesions	Malpighian follicles	R.E. Proli-:feration	Sinus Pro-:liferation	Congestion = C Haemorrhage = H	Inflammatory infiltration	Remarks Type of Reaction
62	5617	Mrs. E.K.	41	Weakness; anaemia; gastro-intestinal symptoms.	426 gm.	-	+	-	+ cuffing	Germ centres	++	?	-	?	Sub-capsular "Fibro-adenie" Type: Proliferative
63	5885	Mrs. C.P.	38	Epistaxia; haemorrhages; menorrhagia.	632 gm.	-	+	-	-	Active	+	+	-	Leucocytic	Type: Inflammatory
64	6028	J.D.	26	Anaemia; splenomegaly.	990 gm.	++	+++ cuffing of vessels	++	- cuffing	Active	++	+	Peri-malpi-:ghian (H)	Leucocytic Lymphocytic	Type: Proliferative congestive and inflammatory
65	6218	J.C.	17	Secondary anaemia; leucopenia; splenomegaly.	1061 gm.	?	+ cuffing of vessels	-	+++ cuffing	Atrophy	+	+++	Trabe-:cular (H)	Lymphocytic	"Fibro-adenie" +++ Type: Proliferative and fibrotic
66	6223	Mrs. G.H.	60	Secondary anaemia; splenomegaly.	480 gm.	+	-	-	+ marked cuffing	No change	+	+++	-	Lymphocytic	"Fibro-adenie" + Type: Proliferative and fibrotic
67	6367	M.	15	Anaemia; headache; yellow complexion.	356 gm.	-	-	-	-	Germ centres	++	?	-	Lymphocytic	Type: early proliferative
68	6894	Miss I.L.	26	Loss of vision in one eye; marked anaemia; leucopenia.	460 gm.	+	+	-	++	Atrophy	+	?	Slight diffuse (C)	Lymphocytic Leucocytic	"Fibro-adenie" + Type: Proliferative and inflammatory
69	7030	Miss M.P.	18	History of jaundice & epistaxis; splenomegaly.	467 gm.	-	+ cuffing of vessels	-	+ cuffing	Germ centres	+	+	Trabe-:cular (H)	Lymphocytic	Type: early proliferative
70	7868	E.		Anaemia; splenomegaly; early cirrhosis.	1195 gm.	++	++	++	+	Atrophy	+	++	(H) Trabecu-:lar; Pulp; (C) Diffuse sinus	-	"Fibro-adenie" ++ Type: Congestive
71	8512	J.H.	8	Splenomegaly.	13.5x9 x 6 cm.	-	+	+	+ Inflam-matory necrosis	Active	++	+++	Trabecu-:lar (H)	-	"Fibro-adenie" ++ Type: Proliferative and early fibrotic
72	8774	G.B.	42	Attacks of haematemesis; splenomegaly.	1370 gm.	++	++	-	+ Hyaline change cuffing	Active	++	++	Trabecu-:lar (H)	-	"Fibro-adenie" ++ Type: Proliferative and fibrotic

TABLE II (Continued)

Serial No.	Specimen No.	Name	Age	Clinical Abstract	Size or weight of spleen	Histology										
						Capsular thickening	Trabecular thickening & splitting	Siderotic nodules	Peri-arter-:ial fibro-:sis & other lesions	Malpighian follicles	R.E. Proli-:feration	Sinus Pro-:liferation	Congestion = C Haemorrhage = H	Inflammatory infiltration	Remarks Type of Reaction
73	9262	Mrs. R.M.	26	Haematemesis; splenomegaly.	338 gm.	++	+ Inflam-:mation	+	+	Atrophy Banti's Fibro-adenie	+	++	Trabecu-:lar (H)	-	"Fibro-adenie" Type: Proliferative and fibrotic
74	D.B. 878	Mrs. M.	44	Splenomegaly; anaemia.	Enlarged	+	+	+	+ cuffing	No change	+	?	-	Leucocytic Lymphocytic Monocytic	Type: Infiltra-:tive (inflammatory)
75	R.I. 606 Vol. 31	D.C.	19	Clinical picture of Banti's disease.	Much enlarged	+	++ Inflam-:mation	-	++ cuffing	Active	+++	++	Peri-follicu-:lar (H)	Lymphocytic Erythro-phagocytes	Sub-capsular "Fibro-adenie" Type: Proliferative and early fibrotic
76	R.I. 246 Vol. 41	Miss I.W.	44	Ascites; splenomegaly; anaemia; leucopenia.	900 gm.	++	++	-	+	Atrophy	++	++	-	Lymphocytic	"Fibro-adenie" ++ Type: Proliferative and fibrotic

COMMENT.

A histopathological study of the behaviour of the spleen in these two groups of cases showed that the reactions met with could be classified under four types. Though in many cases these were not pure and distinct type reactions, they indicated more or less the trend of the histological process. These types were as follows:

Type I. A proliferative reaction of the reticulo-endothelium and of the sinuses.

Type II. An inflammatory reaction with the formation of a macrophage tissue and a leucocytic response.

Type III. A congestive reaction that was common with portal decompensation and the later stages of cirrhosis.

Type IV. A chronic reaction with fibrillary increase either of the capsulo-trabecular system or of the pulp cords.

Type I. The Proliferative Type of Splenic Reaction.

Under this type are included (a) proliferative changes affecting the reticulo-endothelium and (b) a special affection of the sinuses called "sinus pro-:liferation".

(a) The reticulo-endothelial reaction was most marked in the pulp cords, but the sinus endothelium was affected to a variable extent. In the early stages/

stages the change commenced in the pale nuclei which lie scattered about in the protoplasmic ground sub-:stance, the whole forming the syncytium of the pulp. In the normal spleen these nuclei are large pale oval almost vesicular structures of an "endothelial" type with one or two indistinct nucleoli and a thin chromatin network; this network is condensed to form a distinct nuclear membrane which is often convoluted on one side. In hyperplasia the chromatin became more prominent, the nuclear membrane regular and more distinct while division was followed by an overcrowding of the syn-:cytial cytoplasmic ground substance with small round regular forms and larger oval forms which had not yet divided (Fig.3 and 4). This "pale nuclear prolifera-:tion" was so marked in some cases that the pulp mesh was indistinct. Side by side with changes affecting the pulp cords the "endothelial" lining of the sinuses showed a similar change. The long oval nuclei of the rod cells became ovoid or rounded and globular and could be seen projecting into the lumen. In some cases the lining wall became studded with heaped up or dividing nuclei so that the appearance resembled a gland tubule in activity. Occasionally the massed nuclei protruded into the lumen like papilliferous buds in an adenomatous cyst. The marginal zone of the malpighian bodies became more distinct and broadened

from/

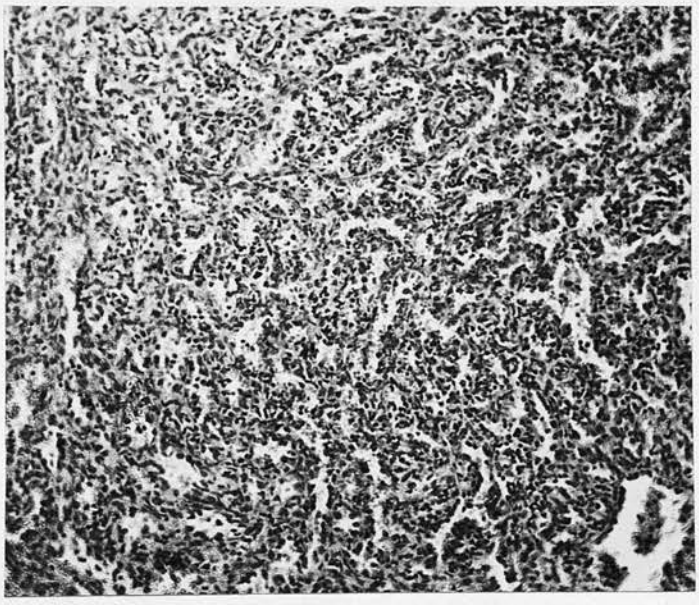

Fig. I . Case No.54. Reticulo-endothelial proliferation in splenic anaemia (x 125) H. & E.

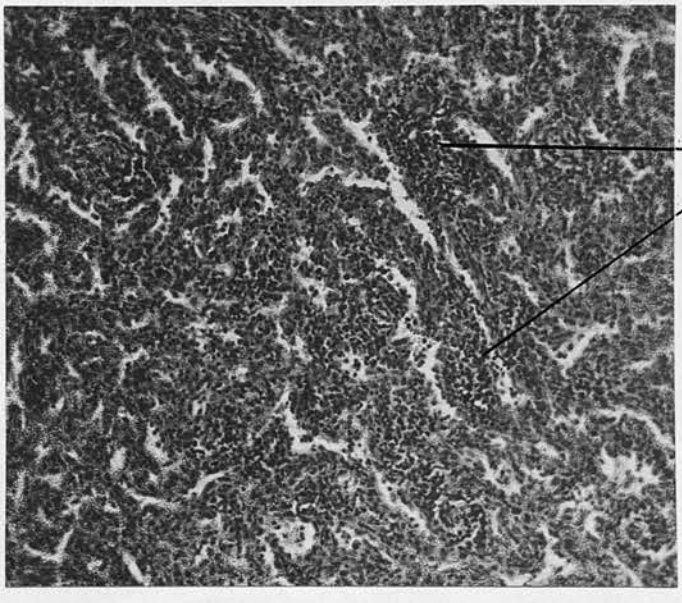

Fig. 12. Case No.66. Reticulo-endothelial proliferation with focal and diffuse lymphocytic infiltration (b). (x 125). H. & E.

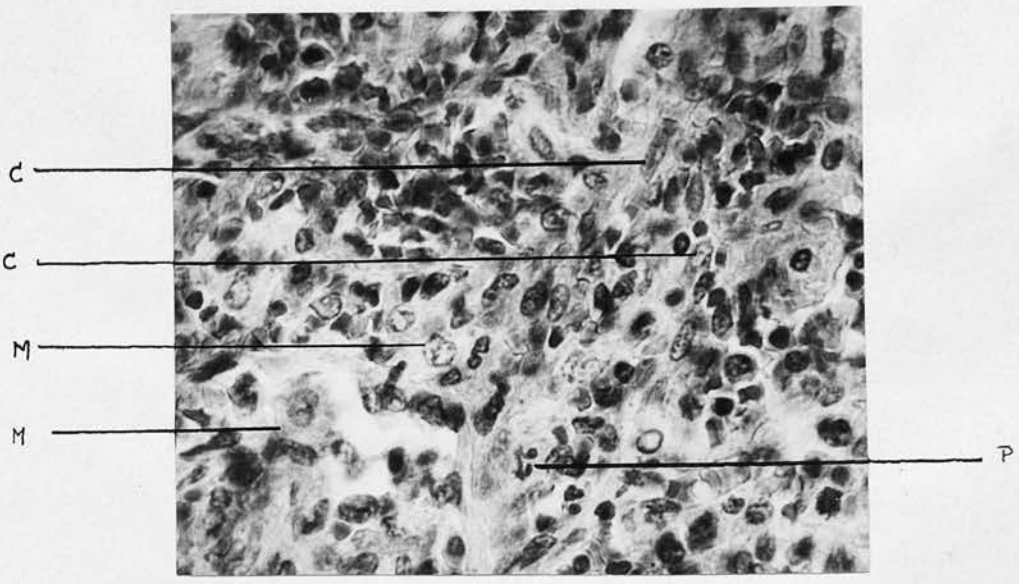

Fig. 3. Case 49. Reticulo-endothelial proliferation and differentiation of macrophages (m. macrophage; c. proliferating pulp cord; p. polymorphonuclear leucocyte). (x 600). H. & E.

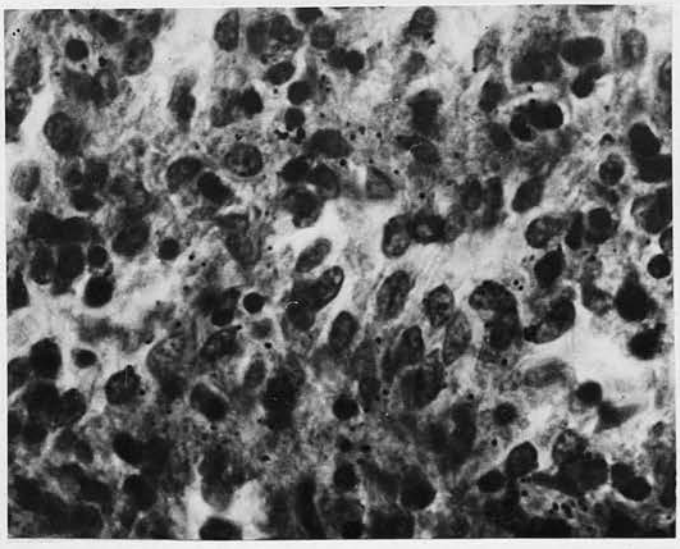

Fig. 4. Case 66. Splenic anaemia showing Reticulo-endothelial proliferation of the pulp cords (x 750). H. & E.

from growth of the reticulum cells. The lymphoid tissue appeared little affected but the sheaths of the penicillar vessels appeared more prominent and hyper-:plastic. Sometimes a similar change was found affecting the lining endothelium, both of the penicilli and of the eccentric arterioles. A gradual increase of the delicate reticulum fibrils of the pulp appeared to follow in the wake of this cellular activity; sometimes the fibrils appeared thick and showed great complexity of branching. Occasionally the change known as "sinus proliferation" was superadded.

(b)"Sinus proliferation" was a characteristic reaction that was occasionally met with. It could easily be made out under low magnification. Here the whole pulp presented a sieve-like appearance from the presence of numerous round or oval rather rigid looking tubular spaces which were lined by cells indistinguish-:able from sinus "endothelium". There seemed to be an alteration and overgrowth of sinus tissue with a corresponding thinning of the pulp cords. The earliest phases could be made out in the meshes of the pulp. Hueck (1928) has demonstrated in the normal spleen, that the mesh consists of irregular gaps formed by the liquefaction of the cytoplasmic reticulum. During contraction of the spleen these gaps become converted into orderly tubule-like structures while in relaxation they/

they revert to their original shape, "the unordered reticulum". In "sinus proliferation" the first change appeared to be a transformation of the pulp spaces into an orderly tubular mesh which seemed rigid and incapable of further change during relaxation. In places, the tubular gaps appeared lined by oval and flattened nuclei of the pulp, these showing a gradual transition to an "endothelial" or littoral cell type (Diagram I and II). The whole change appeared very similar to the formation of capillary spaces by vacuo-:lation and growth of an endothelial lining in the haemangioblastomas (Figs. 5 and 6). These spaces varied in size were sometimes very small sometimes dilated, rounded oval or pyriform in shape and most often empty. Engorgement was not common, but occa-:sionally red blood cells and free littoral cells were present in the lumen. The sinus walls were unusually distinct and the lining nuclei rounded or oval and often protruding into the lumen, suggesting the appear-:ance of a gland tubule. The appearance was quite distinct from that met with in venous congestion where the veins and sinuses appear turgid and thinned out. The nuclear changes suggested some chronic stimulus causing proliferative growth of the littoral cells. This peculiar sinus effect appeared to be the result

of/

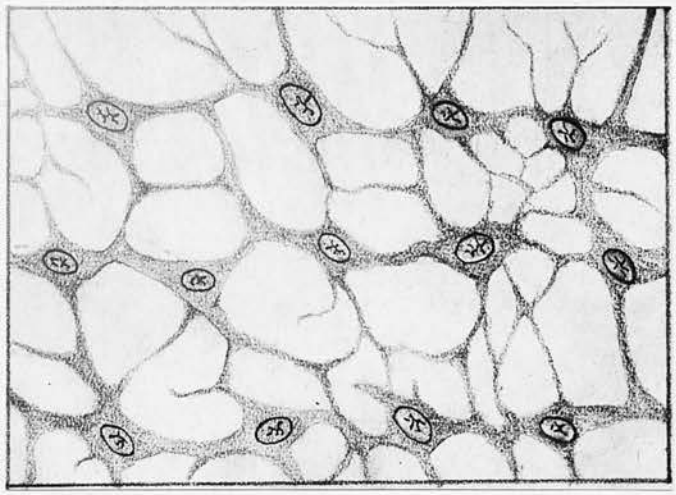

Diagram I (after Hueck) showing the structure of the pulp syncytium and the cytoplasmic mesh; note that the mesh is made up of irregular gaps in the cytoplasm.

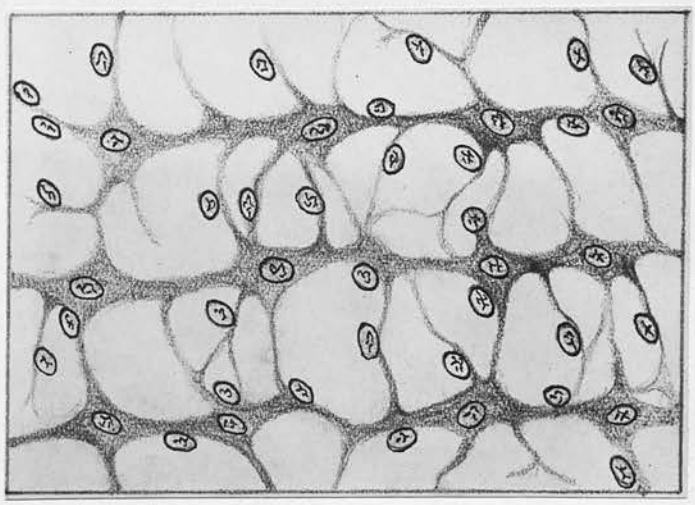

Diagram II showing the commencement of sinus proliferation; there is a nuclear proliferation and the differentiation of an endothelial lining in the pulp spaces.

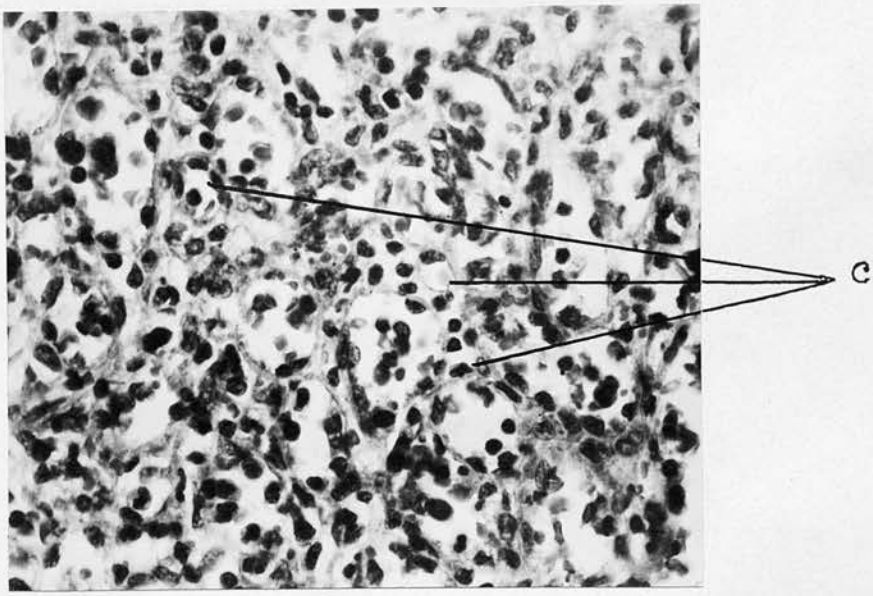

Fig.5. Case No.18. Sinus proliferation showing the formation of small capillary spaces (c) in the pulp cords (x 500). H. & E.

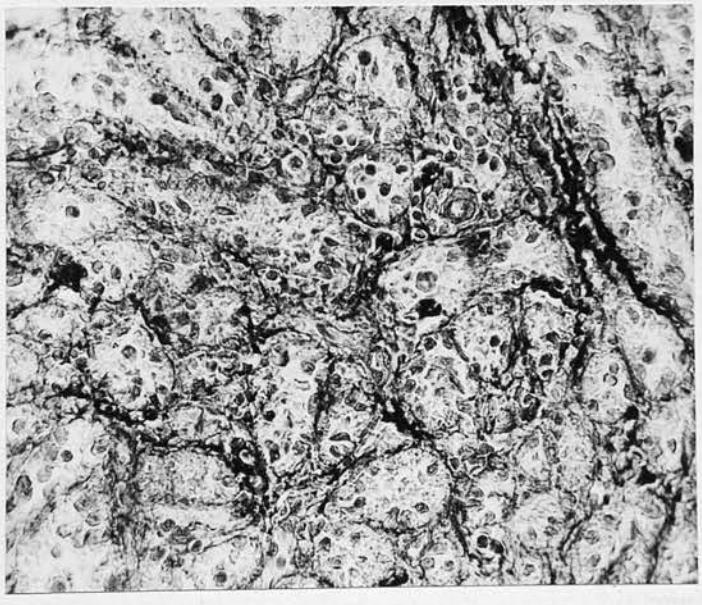

Fig.6. Case No.18. Sinus proliferation showing the littoral cell reaction and the condensation of reticulum in the pulp (x 350). Azan.

of two factors, one a gradual fibrillary increase in the pulp so that the pulp spaces became rigid and converted into tubular structures, and the other a proliferative sinus reaction. The multipotent powers of differentiation of the primitive mesenchymal tissue suggest that this alteration into a more specialised sinus endothelium is probably an adaptation to an altered function.

Type II. The Inflammatory Reaction.

A type of splenic reaction that was frequently met with in early cirrhosis and in splenic anaemias was similar to what is met with in the spleen in infective processes and toxic conditions. Acute leucocytic reactions as in sepsis were uncommon except in cases with complicating terminal infections. Subacute and chronic reactions as in subacute endo-:carditis were more common. All the grades of inflam-:matory response had the one common basis of differen-:tiation of the fixed mesenchymal tissue of the spleen into a free macrophage tissue showing varying powers of phagocytosis. The histiocytes were mobilised by a process of differentiation of the cytoplasmic reticular syncytium and the littoral cells. An associated hyperplasia of the fixed reticulo-endothelium was marked in the more chronic reactions. Previous studies (Part I, (chapter/

(chapter III) have shown that this proliferation reaches its maximum in infections like malaria where the parasitic stimulus remains in the tissues in a state of sub-clinical infection for a considerable time. The common type of cell response in cirrhosis and splenic anaemias was mostly lymphocytic with varying monocytic and plasma-cytic reactions. Leuco-:cytic infiltrations were occasionally met with in relation to necrotic foci in the pulp and around the vessels. The lymphocytes were in clusters and in much larger collections than in normal spleens and an inter-:mingling of plasma cells and monocytes gave the im-:pression that a process of subchronic inflammation was present. That this reaction has no definite relation to inflammatory terminal infections is shown by the accompanying table where both groups are shown (Table III).

Besides those cases where the general pulp reaction was definitely inflammatory, many of the other cases especially in the group of splenic anaemias showed another significant change. This was the tendency of the inflammatory cells to group themselves in clusters around the smaller penicillar arterioles (Fig.8 and 9). This perivascular cuffing was exactly similar to that met with in other organs in chronic inflammation, but has long escaped recognition in the spleen owing to the lymphoid/

lymphoid tissue that is normally present. Even in the malpighian follicles the presence of inflammatory foci around the vessels could be recognized if care was taken to distinguish the monocyte, the plasma cell and the leucocyte that are present in such foci. A careful cytological study of the spleens in cirrhosis and splenic anaemias by a cytoplasmic stain such as Leishman's has brought to light significant peri-:vascular reactions that are mostly inconspicuous in the spleen owing to its extreme cellularity. In cases where the general splenic reaction was frankly inflammatory the perivascular reaction was often extreme.

Another characteristic change was the presence of definite trabeculitis or trabecular infiltration most marked around the trabecular vessels (Fig.10). In some cases, the inflammation was so marked that small capillary vessels were formed in these inflam-:matory foci and these appeared surrounded by clusters of plasma cells and lymphocytes as in a granulation tissue.

Small areas of focal necrosis were occasionally found in the pulp, around the smaller vessels, very often in malpighian follicles (Fig.11) and sometimes in the trabeculae. These were common in cases of acute and subacute yellow atrophy where the necrotic patches/

patches were sometimes obscured by haemorrhage. Sometimes the whole pulp was affected, groups of syncytial nuclei showing karyolysis and pyknosis (Fig.12). It seems possible to correlate these more acute toxic reactions to milder and chronic inflamma-:tory changes in the spleens in cirrhosis as represen-:ting the beginning and the end result of the same morbid process.

Type III. The Congestive Reaction.

This type of reaction was common with well developed cirrhosis and portal stasis. Most often it was associated with capsulo-trabecular thickening and the beginning of fibrillary increase in the pulp. In most cases, the congestive reaction was associated with diffuse inflammatory foci in the pulp and a vary-:ing degree of reticulo-endothelial reaction, so that here all these types seemed to merge imperceptibly. Besides capsulo-trabecular thickening, the characteris-:tic feature was an engorgement of the entire venous system of the spleen. The veins of the trabeculae, the pulp veins and the venous sinuses were all distended with blood and their walls thinned and stretched. Occasionally, recent haemorrhages into the capsule, the subcapsular tissue, the trabeculae, and around the follicles were present. Often the haemorrhages were at the junction between the trabecular veins and the pulp/

TABLE III.

Spleens showing inflammatory reaction.

Serial No.	Group	Type of Reaction.	Complication.
1.	Subacute liver atrophy	Proliferative and inflammatory	Bronchitis
5.	Cirrhosis	Congestive and inflammatory	Acute streptococcal peritonitis
10.	Cirrhosis	Inflammatory and fibrotic	nil
11.	Cirrhosis	Inflammatory and fibrotic	nil
12.	Cirrhosis	Inflammatory and congestive	nil
13.	Cirrhosis	Proliferative and inflammatory	Pneumonia
15.	Cancer gall bladder	Inflammatory	Malignant peritonitis
22.	Acute yellow atrophy	Congestive and inflammatory	nil
25.	Cirrhosis liver	Inflammatory and fibrotic	nil
28.	Cirrhosis	Congestive and inflammatory	nil
29.	Cirrhosis	Inflammatory	nil
30.	Cirrhosis liver	Congestive and inflammatory	Gangrenous appendix removed; purulent bronchitis
34.	Cirrhosis	Proliferative and inflammatory	Bronchopneumonia
36.	Cirrhosis	Congestive and inflammatory	nil
37.	Acute yellow atrophy	Congestive and inflammatory	nil

40/

TABLE III (Continued).

Serial No.	Group.	Type of Reaction.	Complication.
40.	Cirrhosis	Congestive and inflammatory	nil
41.	Cirrhosis	Congestive and inflammatory	nil
43.	Cirrhosis	Inflammatory and proliferative	Lobar pneumonia
48.	Cirrhosis	Proliferative and inflammatory	Pneumococcal peritonitis
49.	Subacute liver atrophy	Proliferative and inflammatory	Septic arthritis; bronchopneumonia
60.	Splenic anaemia	Proliferative and inflammatory	nil
63.	Splenomegaly and haemorrhages	Inflammatory	nil
64.	Splenic anaemia	Proliferative congestive and inflammatory	nil
68.	Splenic anaemia	Proliferative and inflammatory	nil
75.	Splenic anaemia	Inflammatory	nil

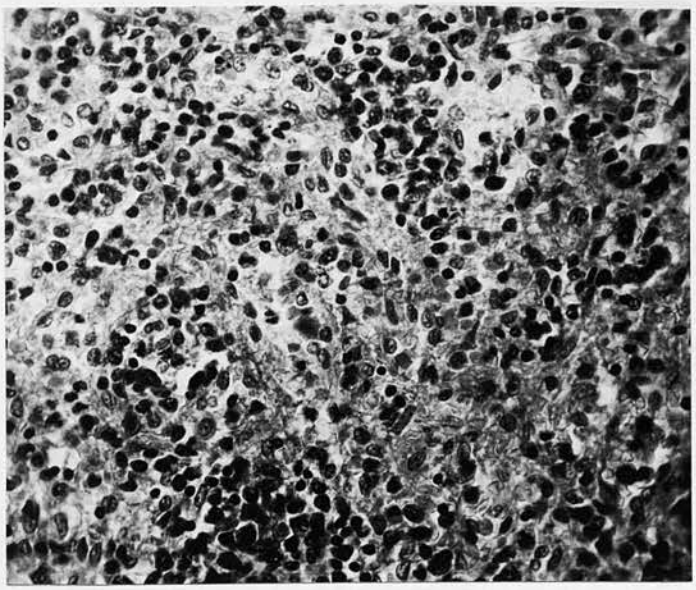

Fig.7. Case No.29. Subacute inflammatory reaction with leucocytic, lymphocytic, and monocytic infiltration (x 350) Leishman.

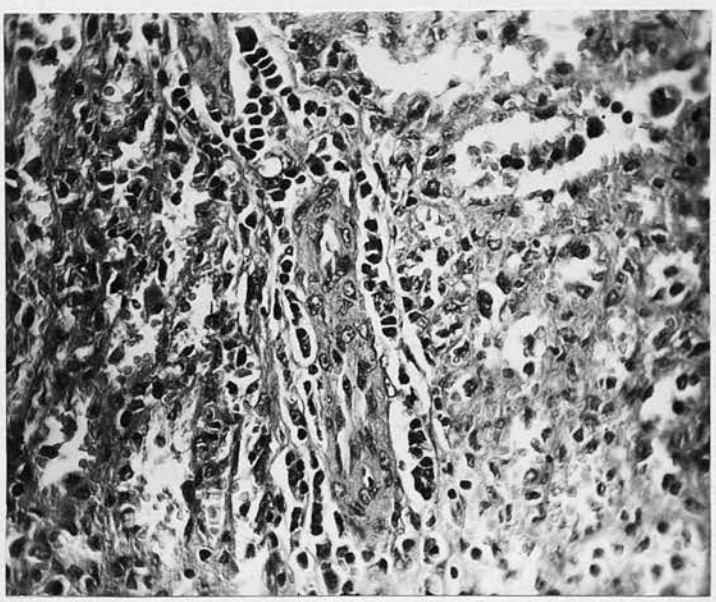

Fig.8. Case 54. Perivascular reaction round a sheathed arteriole (x 350) Leishman.

Fig.9. Case 67. Perivascular cuffing (p) round the penicillar arterioles and capillaries in the pulp in splenic anaemia (x 150). H. & E.

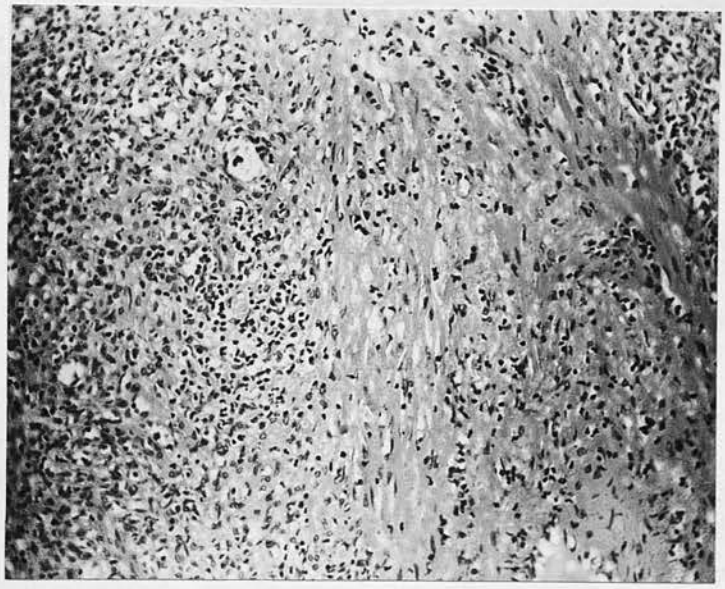

Fig.10. Case 58. Focus of trabecular inflammation in splenic anaemia (x 150). H. & E.

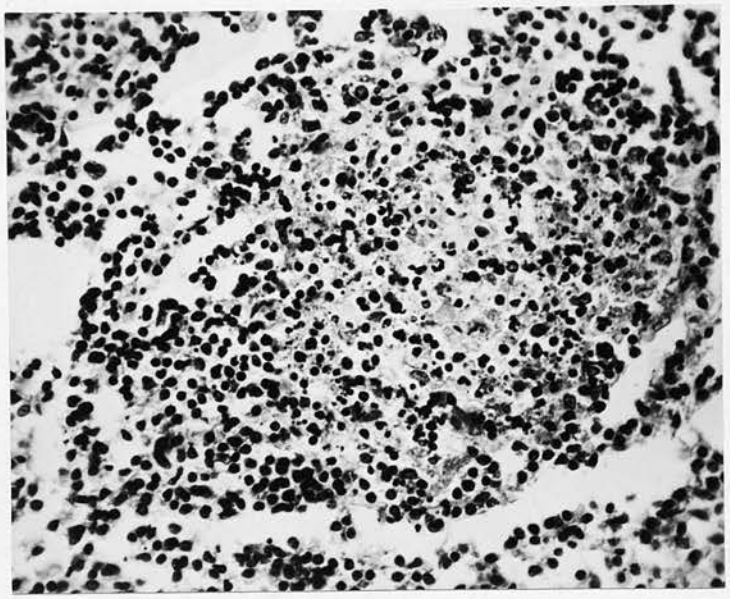

Fig.11. Case No.28. Necrotic reaction in a malpighian follicle in a case of cirrhosis (x 300). H. & E.

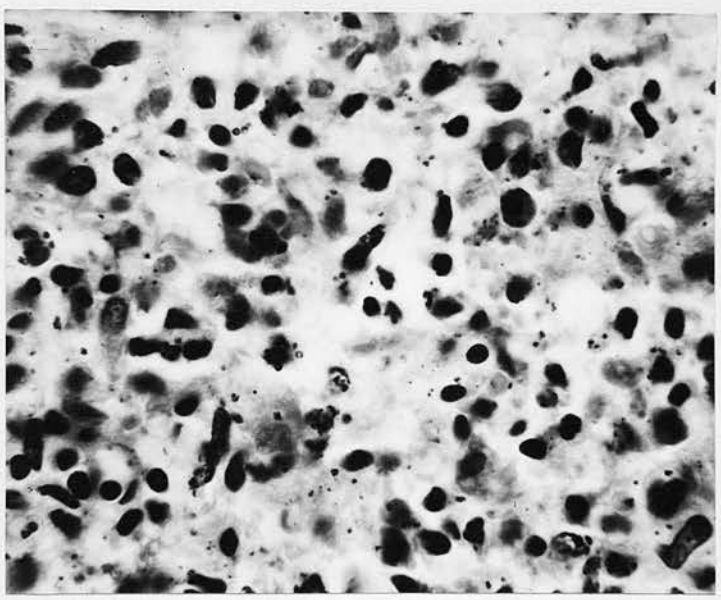

Fig.12. Case 1. Diffuse necrosis of the syncytial nuclei of the pulp in a case of subacute liver atrophy (x 800). H. & E.

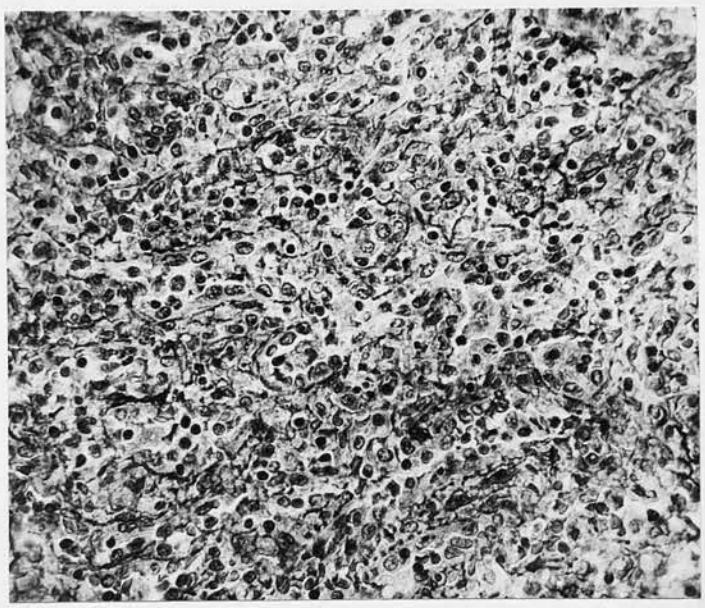

Fig.13. Case 15. Subacute inflammatory reaction with differentiation of the reticulo-endothelium into macrophages. Note the fibril formation (x 350) Azan.

pulp veins. Perimalpighian congestion was common, but no special leakage could be demonstrated at the junction between the arterial and the venous tree around the ellipsoid though this has been suggested by McNee (1929) and McMichael (1934) as the critical point of the splenic circulation. Congestion at the perimalpighian zone would be a natural sequence of increased venous pressure as the sinusoidal tissue of the spleen becomes altered to an annular loose reti-:cular mesh where the follicular capillaries open out at the margin of the follicle.

The engorgement of the sinuses was most marked in the subcapsular and peritrabecular regions where they were distended and thinned out and appeared like cavernous tissue. Towards the centre of the "splenic lobule", distension was not so marked, but the conges-:tion was more diffuse, the blood was not confined to the sinuses alone, but had percolated into the pulp. This appearance is in keeping with the idea of a two fold pathway of the splenic circulation, since the arterial blood is believed to pass through the straight penicillar vessels directly to empty into the sub-:capsular and peritrabecular sinuses at the periphery of the splenic lobule, while in the medullary part of the lobule, the perimalpighian zone, there is the indirect passage through the ellipsoids into the pulp. In consequence, with an increased venous pressure, the blood/

blood would be retained in the pulp mesh in the centre of the lobule, while at the periphery, the sinuses would show distension. The diffuse perimalpighian congestion can thus be better understood as a natural sequence of venous stasis than on the idea of a rupture at the ellipsoid.

Diffuse haemorrhages were frequently met with in the pulp and in some cases these were in the subcap-:sular and peritrabecular region suggesting that the venous sinuses had given way. Haemorrhage into the follicle was exceptional, and in cases where blood was found within, there was a possibility of percolation from the marginal zone. Haemorrhages in the spleen are so frequently the result of toxic capillary damage that by themselves they do not suggest venous obstruc-:tion.

Siderotic nodules were met with in 14 cases of the cirrhosis group and in 10 cases of the group of splenic anaemias. They were most numerous in a case of cirrhosis with acholuric jaundice. They were more frequent in the larger trabeculae (Fig.16). Sometimes they were in relation to pre-follicular arterioles, but rarely in relation to penicilli. They were also more frequent in cases of gross splenomegaly where trabecular splitting and fibrosis had commenced. Occasionally small areas of necrosis and haemorrhage could/

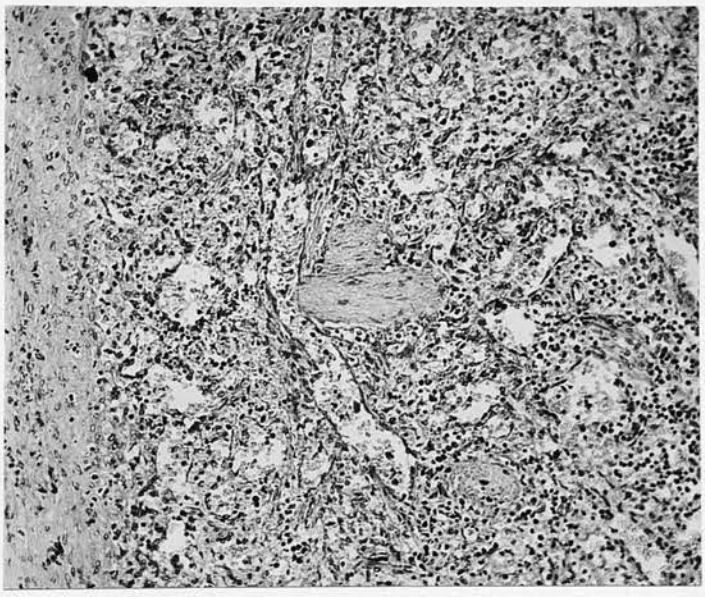

Fig.14. Case No.23. Subcapsular sinus dilation with engorgement. (x 125). Leishman.

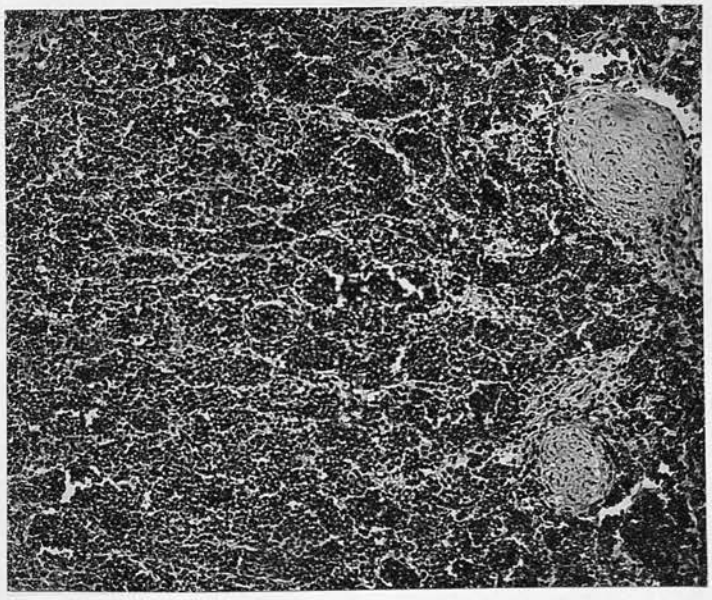

Fig.15. Case No.39. Advanced sinus distension in the congestive reaction. The red blood cells appear black and the sinus walls pale in the section. (x 125). H. & E.

could be made out in relation to the trabecular arteries suggesting a rupture into an area of trabecular softening. Dürr (1924) and Eppinger (1920) have argued that they are the result of increased venous pressure within the trabeculae, but the presence of the nodules in various types of splenomegalies as recorded by Jaffé and Hill (1920) suggests some other factor. Wohlwill (1925) argued that they were produced by rupture of arterioles and McNee (1929) and McMichael (1931) held that the smaller penicillar vessels under-:went degeneration with consequent rupture. Whatever be the site of the rupture, the presence of swollen elastic and collagen fibrils often impregnated with salts of iron, suggests that the haemorrhage was into pre-existing fibrous and elastic tissue. A mere haemorrhage into the spleen pulp is followed by organisation as in an ordinary red infarct, and is not followed by the appearance of longitudinal bands of tissue incrusted with iron. The picture is that of haemorrhage into preformed fibrous and elastic tissue as in the trabeculae, possibly into an area of peri-vascular softening or inflammation. The occurrence of foci of trabeculitis around the vessels points to a local damage of the vessels wall rather than a mechanical stress.

The malpighian follicles were generally atrophied. This/

This has been looked upon as the effect of increased distension of the pulp, but the peri-vascular fibrosis suggests the possibility of a chronic follicular inflammation. Little evidence of hyperplastic activity could be made out. Germinal centres were mostly inconspicuous and the cell type was small lymphocytic. The eccentric arterioles and their smaller capillary branches showed in the large majority of cases an increase of the adventitial reticulum as compared with the normal. In some cases the muscle coat appeared fibrotic. The penicillar vessels of the pulp showed a similar increase. Well marked fibrotic follicles resembling Banti's follicular "fibro-adenie" were rare. Haemosiderosis was not well marked in the cirrhosis group but more common in the group of splenic anaemias.

Hyperplastic changes in the sinus wall were not common in this group; the littoral cells appeared stretched out and the nuclei sparse in distribution. A slight compensatory increase in the fibrillary reticulum was noted.

Cellular infiltrations were not so marked as in the inflammatory type described above. A low grade of inflammatory response was found in many cases besides those shown in Table III . Megakaryocytes were seldom met with and immature cells of the myeloid series/

series were rare. Degenerative changes in the reticulum cells and the mononuclear cells were found in a few cases, but were more marked in the inflamma-:tory type described. Infiltration with fat globules and lipoid droplets was noticeable in a few cases, but was never marked.

Type IV. Fibrillosis; Fibrosis; "Fibro-adenie".

Marked capsulo-trabecular thickening was an occasional feature in the congested spleens of cirrhosis (Fig.15), but the change was more or less localised and had not caused a diffuse fibrosis of the pulp. A hyaline perisplenitis causing extreme thick-:ening of the capsule was exceptional. In such cases the true capsular tissue could be made out under the hyalinised outer layer. A type of fine reticular sclerosis or fibrillosis was more characteristic of the large spleens whether in cirrhosis or in pre-cirrhotic splenic anaemia and especially in those cases which gave a clinical picture of Morbus Banti. "Fibro-adenie" was the term first suggested by Banti (1894 and 1898) to describe this fine fibrillary increase and adenoid appearance of the cellular tissue in the lymph follicles and pulp cords. He held that the change was different from the chronic inflammatory fibrosis as there was no fibroblastic growth, but an increase of the finer fibrils which gradually became thicker/

Fig.15. Case No.27. Extreme capsulo-trabecular thickening in a congested cirrhotic spleen (x 6). Azan.

Fig.16. Case No.35. The distribution of siderotic nodules along the trabeculae (T) down to the follicular arteries (x 6). Leishman. Note that the extent of the lesion in the trabeculae suggests a tracking of blood from the trabeculae down to the small arterioles rather than a reverse process.

thicker and more complex so that the pulp mesh was narrowed. He laid great stress on the follicle reaction where the change commenced from the central artery and spread peripherally towards the margin of the follicles so that the lymphoid elements gradually disappeared. The follicles were not all simultaneously affected but successively so that various stages of this peri-arterial fibrosis could be made out in the same spleen. This he regarded as the essence of the anatomical picture of the Banti spleen and denied (Banti, 1906) that such follicle reactions were met with in the spleens in liver cirrhosis.

Matsui (1915) in his studies on the reticulum fibrosis could only find a difference in degree between the fibrillary increase in cirrhosis spleens and the spleens in Banti's disease. In the present investigation it was noticed that two types of fibril-:lary increase could be made out. In the proliferative reactions in the later stages of splenic anaemias and in a few cases of liver cirrhosis the remarkable feature was the uniform fibrillary increase in the pulp cords which had become altered in pattern owing to alteration of the pulp syncytium. The whole pulp appeared riddled with small round openings of variable size representing what seemed to be newly formed sinuses; the fibrillary increase was in the pulp tissue surrounding/

surrounding these sinuses. With the Foot-Wilder stain the appearance was that of a fine lace work with small almost regular round or oval openings formed by the sinuses (Fig.23). This uniform "fibro-adenie" of the pulp was quite distinct from the type of fibrillary increase spreading from the capsule and the trabeculae, found in various types of chronic splenomegaly and even in the senile spleen where Gauckler (1904) described it as the "sclerose-atrophique" reaction. In many cases the "fibro-adenie" could be seen to spread from the small penicillar vessels as a peri-capillary fibrillosis and also from the vessels of the follicle as a peri-arterial change. The change could be well demonstrated in its entirety in cases where the smaller capillaries showed a concomittant hyaline change as in Case 58. This reaction seemed to differ only in its development from the inflammatory fibrosis met with in other organs in that there was little formation of fibroblastic granulation tissue. Chronic inflammatory cell infil-:trations however appeared side by side with a gradual increase in the reticulum. The reticulum fibrils retained their structure for a considerable time and transformation into collagen was slight and only marked in areas where collagen fibers could spread from the trabeculae or capsular tissue. Sometimes a process of fibro-blastic differentiation seemed to have taken place/

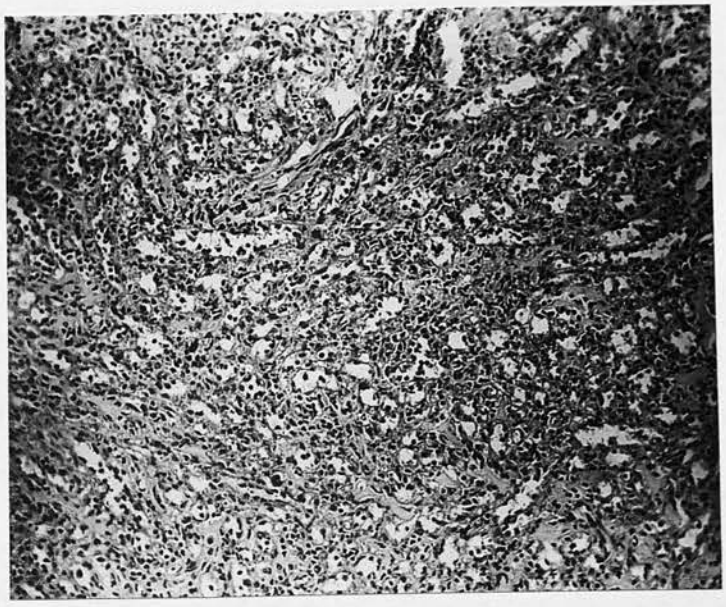

Fig.17. Case No.53. "Fibro-adenie" of the Banti type in the pulp; the sinuses appear narrowed, small and numerous. (x 125). H. & E.

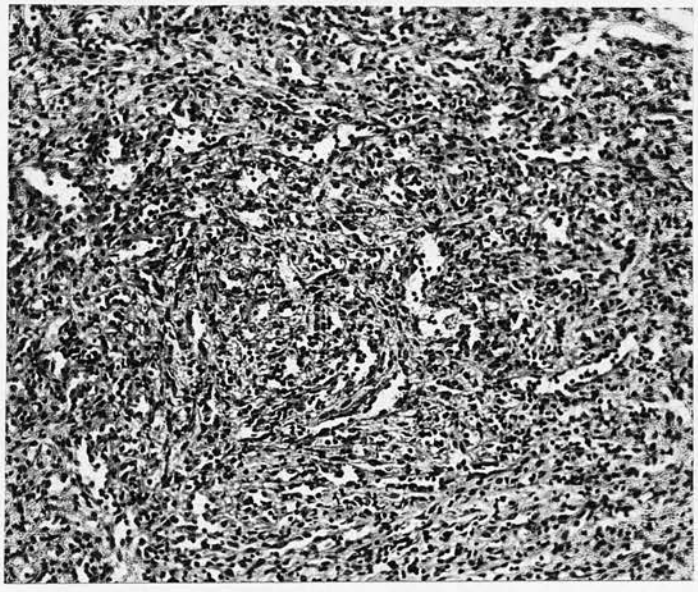

Fig.18. Case No.52. "Fibro-adenie" of the pulp at a later stage with collagenisation and thickening of the pulp cords. (x 125). H. & E.

Fig.19. Case No.49. Early peri-arterial fibrillary increase in subacute liver atrophy. (x 125). Azan.

Fig.20. Case No.42. Fibrillary increase round the follicular and prefollicular arteries in cirrhotic spleen. (x 125). Azan.

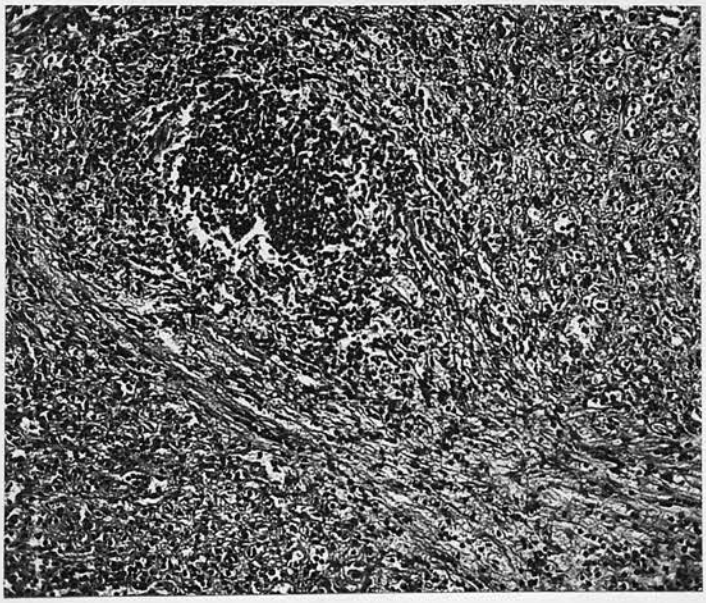

Fig.21. Case No.52. Banti's follicular "fibro-adenie". (x 125) In a case of splenic anaemia. H. & E.

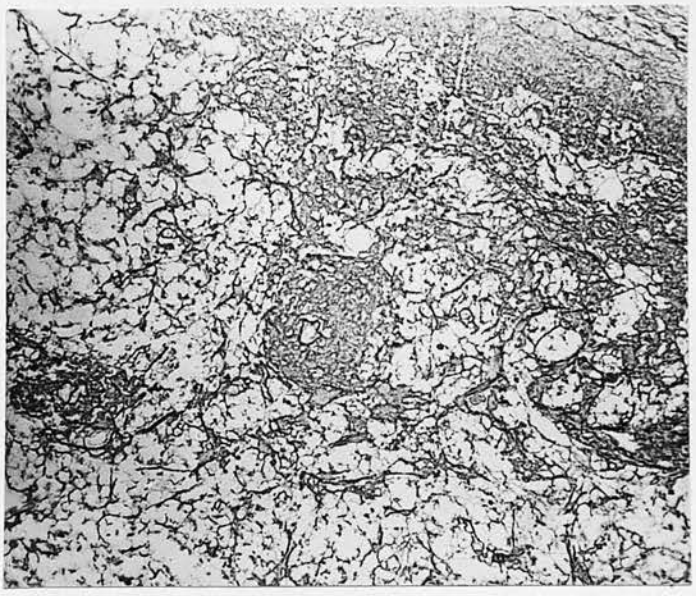

Fig.22. Case No.35. Subcapsular fibrillary increase with irregular collagenisation in cirrhotic spleen. There is no sinus proliferation. (x 125). Foot-Wilder.

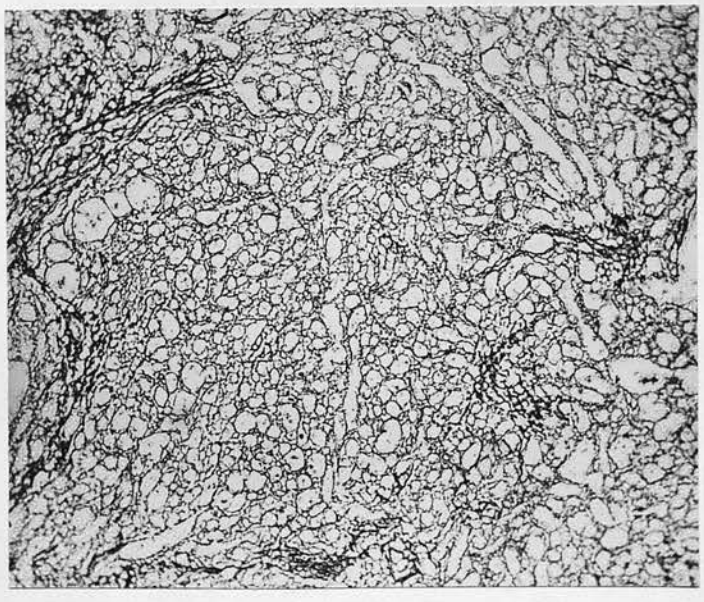

Fig.23. Case No.52. Reticular increase with sinus proliferation of the Banti type (x 120) Foot-Wilder. Note the uniform fibrillary increase in the pulp and the sieve-like appearance caused by the formation of small sinuses.

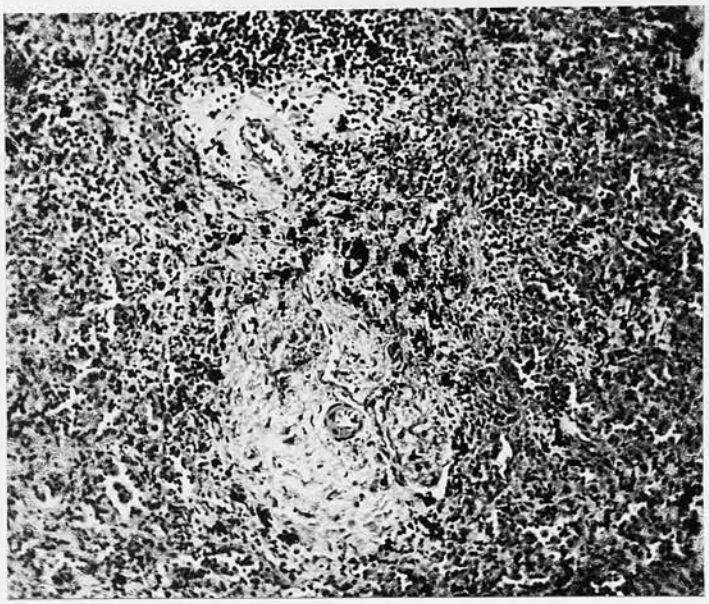

Fig.24. Case No.4 (malarial series). Typical Banti's follicular "fibro-adenie" in chronic malaria. (x 125). H. & E. There is no suggestion of peri-arterial haemorrhage.

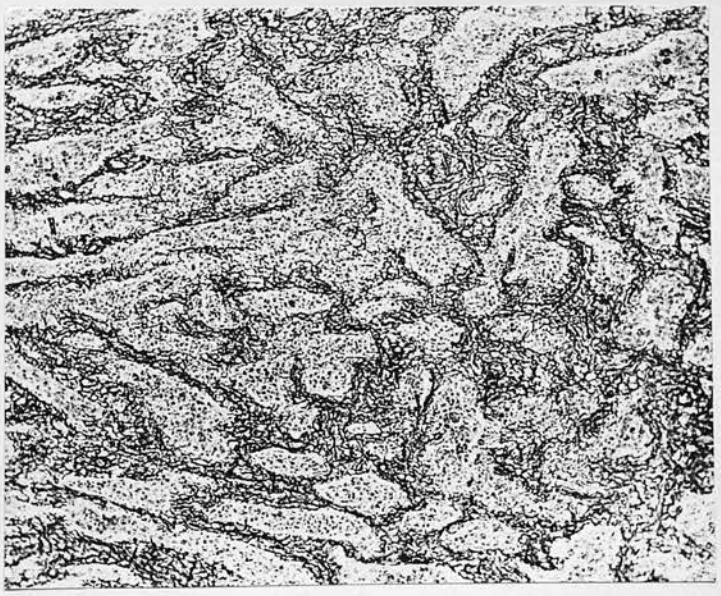

Fig.25. Case No.4. (malarial series). Marked fibrillary increase of the pulp with hyperplastic sinus reaction in chronic malaria. (x 120). Foot-Menard. Note the sinuses appear wide and the pulp is condensed in between.

place in the pulp nuclei which had become elongated and more prominent and lost their endothelial pattern.

Bengal Splenomegaly.

De (1932) in an analysis of splenomegalic conditions in Bengal has drawn attention to a type that was neither malarial nor due to kala-azar, but ran a clinical course that was more or less similar to Banti's disease. De described the disease as having a regional distribution in Bengal, a chronic course of several years, irregular fever, a secondary anaemia and a progressive splenomegaly associated with an enlarged liver which in some cases became cirrhotic in the late stages. The splenomegaly was often extreme with weights from 1000-3000 gm. Chatterji (1934) described the haematological picture as that of a chlorotic type of anaemia with the red cell count varying from two to three millions and a leucocyte count of two to four thousands. The van den Berg reaction was generally "negative" and sometimes "delayed positive". The fragility of the red blood cells was normal. The aldehyde and stiburea tests for kala-azar were negative. Histologically, De (1932) has described a cellularity of the pulp, an atrophy of the malpighian follicles, a thickening and fibrosis of the trabeculae and the presence of large phagocytic cells in the spleen pulp.

Of the material studied three specimens were from cases/

cases in the early stages of the Bengal type of splenic anaemia obtained at operation while the last was from a case with early cirrhosis obtained at autopsy. The histological picture showed a moderate capsulo-trabe-:cular thickening, trabecular splitting and early fibrotic changes in the pulp. The cords of Billroth showed a marked reticulo-endothelial proliferation and a gradual fibrillary increase that was most marked in the most chronic of the series. The transitions from plump oval nuclei to an elongated deeply staining type with the gradual fibril formation, were clearly shown. Well defined signs of chronic inflammatory reaction were evidenced by focal clusters of lymphocytes, mono-nuclear cells and plasma cells. The littoral cells lining the sinuses also showed hyperplastic changes, but the most remarkable feature was the presence within the sinuses of large round mono-nuclear cells often between 40 and 50 μ in size and loaded with clusters of red blood cells. These were not megakaryocytes nor multinucleated giant cells, but large erythrophagocytes somewhat like those met with in typhoid fever. Each had a round nucleus which was often indistinct and the cytoplasm was loaded with ten to twelve red blood cells in various stages of digestion. There was no evidence of malaria or kala-azar.

Changes in the splenic vein.

In view of the discussion regarding the importance of/

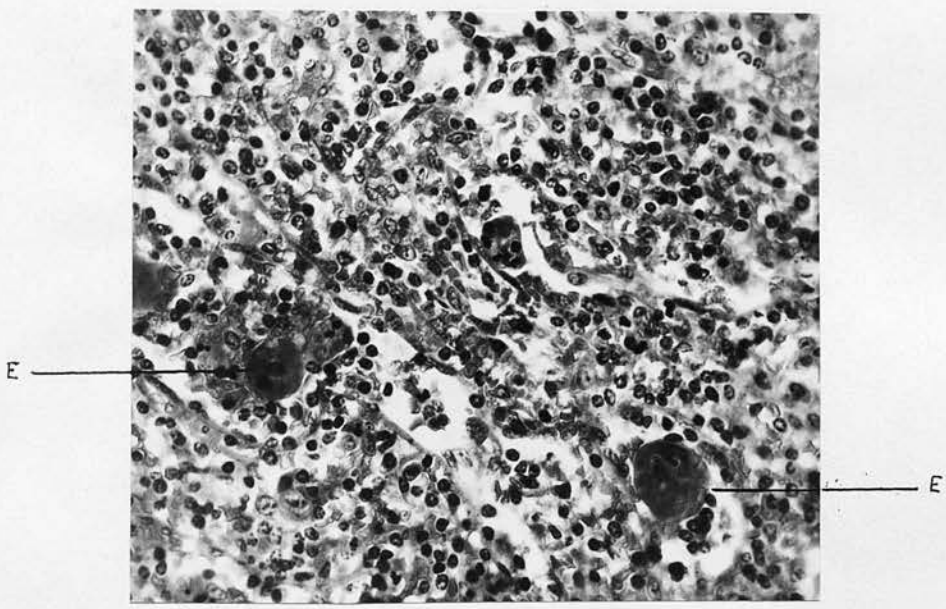

Fig.26. Case No. Bengal splenomegaly. Reticulo-endothelial proliferation, cell infiltration and the formation of giant erythrophagocytes (E) (x 350). H. & E.

of changes in the splenic and portal vein in the pro-:duction of the splenic anaemia syndrome, it was decided to examine the splenic vein in all specimens available. The result of histological examination is summarised in Table IV. It will be noticed from this table that the only frequent change that was present was a slight intimal thickening passing on to endo-:thelial degeneration very similar to atheroma (Fig. 27). The connective tissue cells in the deeper layers of the intima gradually became swollen and stellate in appearance so that the whole tissue appeared to have undergone a myxomatous degeneration. In one case areas of calcification were present in the deeper layers. There was no evidence to suggest thrombosis. In one case the splenic vein at the hilum appeared to have undergone cavernous transformation similar to what has been described in the portal vein by Klemperer (1928).

The subintimal sclerosis and degeneration was quite patchy in distribution and could not be regarded as an obstructive lesion since the lumen of the vessel was quite wide. It is also difficult to imagine that such a patchy subintimal thickening would bring about a chronic and extreme splenomegaly. On the other hand these changes could be more reasonably regarded as secondary to some alteration in the blood that passed through/

through the splenic vein. In chronic malaria where the splenic vein is filled with pigmented macrophages and parasites such changes in the vein are quite common (see Fig.28).

TABLE IV.

Changes in the Splenic Vein in Splenic Anaemia.

Serial No.	Specimen No.	Changes in the vein.
53	4637	Patchy subintimal thickening; early stage.
54	4938	Patchy subintimal thickening.
55	4940	Patchy fibrosis and oedema of intima; subintimal degeneration.
56	5094	Slight hypertrophy of the muscle coat.
62	5617	No abnormality.
65	6218	Subintimal thickening like atheroma; patchy calcification in subintima.
66	6223	Slight hypertrophy of the muscle coat.
67	6367	No abnormality.
68	6984	Patchy thickening of the subintima.
69	7030	Patchy thickening of the intima.
70	7868	Patchy subintimal thickening and degeneration.
71	8512	No abnormality.
72	8774	Marked hypertrophy of the wall to 5 times the normal; change in subintima like atheroma.
73	9262	Cavernous angiomatous trans-:formation of splenic vein at hilum.

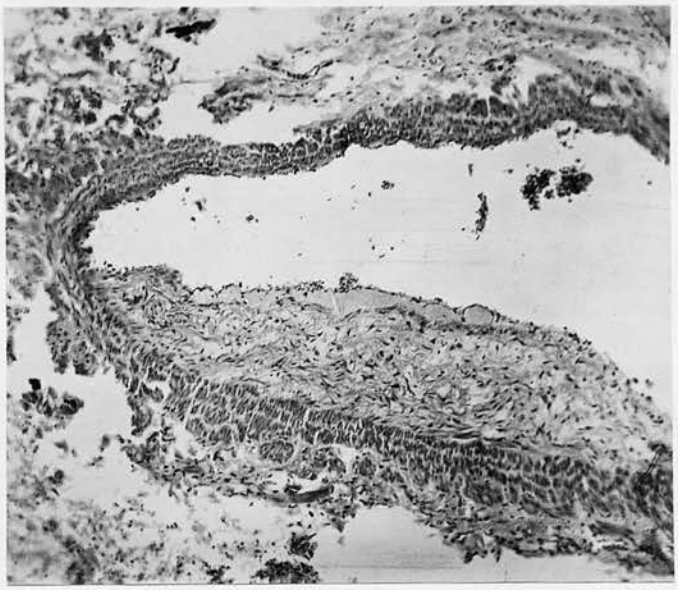

Fig.27. Case No.54. Showing the subintimal thickening and degeneration of the splenic vein in splenic anaemia. (x 60). H. & E.

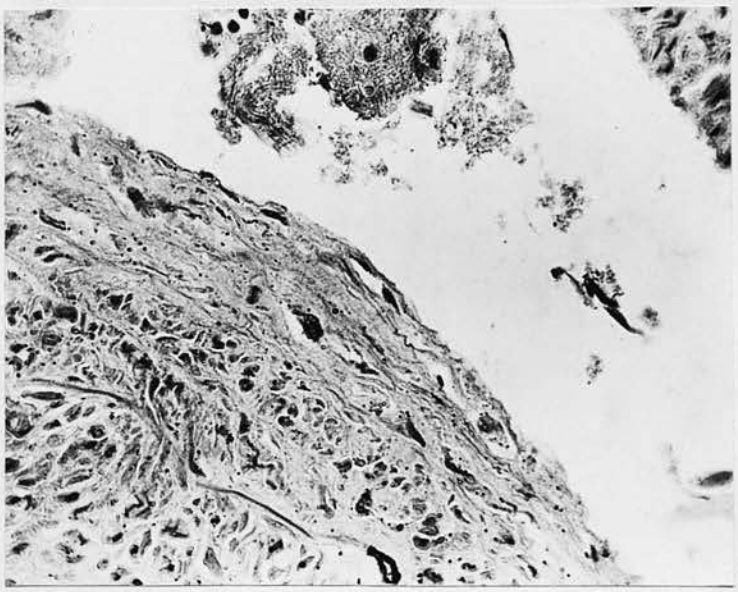

Fig.28. Case 4. Malarial series. Showing patchy intimal thickening in the splenic vein in malaria. (x 300). H. & E.

DISCUSSION.

The Significance of the Peri-vascular Fibrosis and "Fibro-adenie".

Banti (1910) regarded the peri-vascular fibrosis and general fibrillary increase as a degenerative change due to some poison in the blood which so altered splenic function that splenotoxins were formed which subsequently caused liver cirrhosis. He denied that the fibrosis was post-inflammatory, but claimed that it was due to the diffusion of toxins from the artery as shown by the peri-vascular change. Dürr (1924) regarded the lesion as the result of a mechanical pull or overstretching of the vessels from increase in size of the spleen. McMichael (1934) held that the fibrosis was the result of old peri-vascular haemorrhages from increased venous pressure in the spleen. He found artificially produced haemorrhages followed by organi-:sation produced a fibrillary overgrowth that was almost indistinguishable. Such haemorrhages are no doubt met with, but it is difficult to account for the varying grades of perivascular fibrosis by such a lesion.

In this connection, a study of the normal reticulum of the spleen is of some significance. The delicate reticulum fibrils which branch and anastomose in "the splenic/

splenic lobule of Mall" (Mall, 1898, 1900) are con-:densed to form a closer mesh in the subcapsular region, in the peri-trabecular zone and around the blood vessels. It has also been demonstrated that under the capsule and around the trabeculae these fibrils appear to form attachments to the collagen fibers. Henle (1895) first pointed out that the distribution of fibrils around the sinuses is peculiar in that here they form only annular encircling bands; the longitudinal strands within the sinuses are not really made of reticulum as described by Hartmann (1930), but of protoplasmic threads of the rod cells which seem to form a syncytium (Foot, 1927). It is not certain whether the reticulum is distinctly extra-:cellular or formed within the cells. In any case the cytoplasmic mesh is so closely followed by the reticular mesh that there seems to be some justifica-:tion for the view that reticulin is a secretory pro-:duct of the pulp syncytium.

In the present investigation it has been possible to demonstrate that the formation of reticulin followed on the wake of a hyperplasia and differentiation of the reticulo-endothelium. This increase in the reticulum fibres appeared confined to the pulp mesh and the spiral fibrils of the sinuses. It seemed doubtful if the sinus cells formed any reticulin at all, as no fibrils could/

could be demonstrated within the sinus wall. If it can be accepted that reticulin is a true secretory products of the primitive mesenchymal tissue, a reticulo-endothelial stimulus as in chronic inflammation would be followed by an increase in the reticulum fibrils as a functional sequence. Histologically as the fibrils increased in number they became thicker and showed increased branching, and under the capsule and around the trabeculae they gradually assumed the appearance of collagen fibers. The frequency of demonstrable chronic splenic inflammation either as a diffuse change or in perivascular or peri-capillary foci, suggests that the stimulus for the fibrillary increase and reticulo-endothelial reaction is a toxic agent that passes through the vessels into the pulp. The uniform "fibro-adenie" of the pulp would point to the diffusion of such an agent. In chronic malarial splenomegaly where there is no suggestion of any complicating portal effect, but an intermittent diffusion of parasites along the blood stream, typical follicular "fibro-adenie" is met with indicating either a toxic effect or a mechanical blockage of the follicular arterial capillaries (see Fig.24). The morphological similarity of the lesion produced by manganese and senecionine in experimental animals is also suggestive in that here perivascular necrosis and foci of inflammation are followed by peri-:vascular/

:vascular fibrosis affecting the follicles as well as the pulp. The presence of focal necrotic lesions in the spleen in acute and subacute yellow atrophy and occasionally in cirrhosis tends to show that the sequence of events in man is more or less similar. From the vessels, there is the diffusion of a toxic agent which, if intense, caused a periarterial necrosis or diffuse necrosis of the pulp, if mild, a chronic inflammatory reaction with fibrosis. The peculiar histology of the spleen with its large collections of lymphoid tissue, with a basic ground substance of un-:differentiated mesenchyme, and its supporting reti-:culum instead of connective tissue, has obscured the picture of a chronic inflammation, that it has escaped recognition.

Primary Thrombo-phlebitis of the Splenic or Portal Vein as the cause of the Splenic Anaemia Syndrome.

The association between splenic enlargement and endophlebitis of the splenic vein was first noted by Bonné (1884) who suggested that it was a continuation of the inflammatory splenitis into the wall of the vein. Borrmann (1897) however described a primary endophlebitis with thrombosis of the portal vein which caused splenomegaly, but Saxer (1907) opposed the view that the endophlebitis was primary. The idea of a thrombo-phlebitic/

thrombo-phlebitic splenomegaly has gradually gained ground and cases were reported by Dock and Warthin (1904), by Oettinger and Fiessinger (1907), by Edens (1907), Goldman (1913) and others. Warthin (1911) suggested that the Banti syndrome of anaemia, splenomegaly and liver cirrhosis was really due to portal thrombosis. Ziegler (1914) denied this definite association. Wohlwill (1925) in his study of 16 cases concluded that portal sclerosis and thrombophlebitis were part of the morbid picture of a process that affected the spleen, the liver and the portal vessels. Similar cases were reported by Klemperer (1928, 1936) who drew special attention to the histological picture of fibro-adenie of the follicles, and the fibrillary increase in the pulp as exactly similar to changes found in Banti's disease. McMichael (1931) who made a careful study of the problem could find no evidence of portal thrombosis in any of his twenty cases of splenic anaemia. He held that the intimal sclerosis and medial hypertrophy that are sometimes found are probably due to portal hypertension, which was associated with microscopic or macroscopic lesions of the liver.

In the present investigation of cases of splenic anaemia no evidence of portal or splenic thrombosis was found. In one case a cavernous transformation had/

had taken place. This could be argued as due to an organising thrombus. The histological analysis of 15 cases is shown in table IV. In two other cases of thrombophlebitis of the splenic and portal veins that came up to autopsy the spleen showed venous infarction with haemorrhage. In chronic thrombophlebitic splenomegaly it is difficult to decide whether the changes in the vein are primary or dependant on chronic toxic processes in the spleen. Barker (1936) has pointed out the association between primary thrombophlebitis of the peripheral veins and focal infections and it seems possible that an endothelial toxin passing from the spleen may cause intimal changes in the splenic or portal vessels. It is also difficult to see how chronic venous stasis could induce reticulo-endothelial hyperplasia since similar organs such as the liver and bone marrow do not show such reactions to congestion. The experimental work on congestion of the spleen is against the view that long standing venous stasis of whatever kind would induce a marked chronic splenomegaly (see Chapter II, Part I).

Hypertrophy and Hyperplasia of the Spleen.

Problems that have to be settled are whether the large spleens met with in splenic anaemia are really hypertrophied/

hypertrophied organs which take on increased functional activity, and whether the anaemia is due to an exaggera-:tion of the process of erythrocyte destruction that is normally taking place in the spleen. It is of interest that McNee (1929) in his classification of the splenomegalies of Great Britain has placed in a separate group simple hypertrophies where there is extreme enlargement with no other alteration in structure. Lubarsch (1927) defined true hypertrophy of the spleen as a growth of the organ in its entirety, but at the same time main-:taining normal intrasplenic relationships. He held that a real hypertrophy of the pulp did not exist as it had no permanent structure. The terms hypertrophy and hyperplasia should not in the strict sense, be applied to conditions where there is any other altera-:tion in structure that can be regarded as morbid. A careful histological study is the only method of approach.

It must be admitted that so far as this analysis goes neither splenomegalic cirrhosis nor splenomegalic anaemia present indubitable evidence of growth and multiplication of the malpighian follicles, though many cases show hyperplastic reactions of the reticulo-endothelial tissue. There seems to be no doubt that in cases of splenomegaly of the Banti type, the normal intrasplenic relationships are no longer maintained, but/

but processes of hyperplasia and even hypertrophy of cells or tissues are quite frequent. These are not hypertrophies or hyperplasias of the spleen as a whole, but pathological types of proliferative growth probably due to a chronic toxic stimulus.

With regard to the question of increased functional activity in these conditions of splenomegaly, there is histological evidence of increased erythrophagocytosis in Bengal splenomegaly and in some cases of splenic anaemia. An exaggeration of the erythrophagocytic function of the spleen may possibly result from toxic damage of the erythrocytes or from an increased functional activity of the spleen. Studies on haemolytic jaundice and acute malaria indicate that when the red cells are damaged there is the possibility of an extracellular destruction in the pulp where the damaged erythrocytes are aggregated by an active hyperaemia of the spleen. On the other hand, an increased erythrophagocytosis possibly indicates an increased functional activity of the pulp cells. The presence of active erythrophagocytes in the spleen pulp in experimental manganese cirrhosis of rabbits is of some significance in regard to the question of the production of a pre-cirrhotic splenic anaemia.

<u>The Significance of the Reticulo-endothelial Reaction.</u>

Could/

Could the proliferative reaction be part of an inflammatory response or could it be regarded as a functional overgrowth of a specialised tissue? A hyperplasia of the reticular syncytium is occasionally met with in some types of acute bacterial infections and virus diseases, but the reactions are often ob-:scured by other changes such as congestion and cellular infiltration. Such hyperplastic reactions in the spleen have been noted in streptococcal and pneumococcal infections (Barbacci, 1895), in bubonic plague (Dürck, 1904) and in small-pox (Councilman, MacGrath and Brinkerhoff, 1904). In experimental streptococcal infections in white mice Louros (1928) found well marked gradations of reticulo-endothelial response in animals that survived. The changes varied from increased staining, hypertrophy and hyper-:plasia of cells to the formation of local nodules. In streptococcal infections in white mice Orr (1932) found a similar reticulo-endothelial proliferation most marked at the peri-malpighian zone. These changes were early and preceded the leucocytic reaction. In chronic infections, this proliferative reaction becomes predominant either in the form of local nodules in the granulomatous disease or as a more diffuse change in protozoal infections such as malaria and kala-azar. Such a reaction was well marked in a case of monkey malaria which had been rendered/

rendered chronic by the administration of quinine (Part I, Chapter III). In such cases the reaction appears to be of the nature of an immunising response to the infection. It is well marked when the infection is chronic but gradually disappears when the infection dies down.

It would appear that the reticulo-endothelial reaction in splenic anaemia and splenomegalic cirrhosis can be regarded as a response to a chronic infective or toxic process which affects the spleen for a considerable time and induces a mild chronic inflammation. Other histological evidence supports this view.

The Relation between Splenomegalic Anaemia and Splenomegalic Cirrhosis.

The lack of relation between the degree of the portal obstruction and the extent of the splenomegaly is a striking feature in the morbid anatomy of liver cirrhosis. Parkes Weber (1897) pointed out that cases of cirrhosis with jaundice showed more splenic enlargement than cases showing gross portal obstruction. It is also well known that cases of splenic anaemia show only slight and often microscopic lesions of the liver. It is difficult to attribute the gross splenomegaly in such cases to the slight hepatitis which can be reasonably regarded as an associated lesion.

So far as the present histological analysis goes,

it/

it would seem that splenomegalic cirrhosis and splenic anaemia have the one common basis of a chronic splenic inflammation. In most cases of old cirrhosis with portal decompensation the inflammatory lesion is to some extent masked by the venous stasis. In cases where the hepatic lesion is slight and early, the signs of focal inflammation in the spleen are in the fore-:ground. The group of splenic anaemias show more or less similar reactions. In the two latter groups proliferative reactions of the reticulo-endothelium are also very frequent. The splenomegaly of liver cirrhosis is thus the result of an independent chronic splenitis together with a dependent venous stasis. This chronic splenitis is even more marked in splenic anaemia where there is a chronic proliferative inflamma-:tion with only slight associated lesions in the liver. It would seem that different toxic agents vary in their behaviour as regards hepato-lienal damage. In experi-:mental work there is a parallel in the behaviour of a chemical poison such as carbon-terachloride which causes all the stages of necrosis, collapse sclerosis and true cirrhosis of the liver in rats with only proliferative reactions in the spleen, in contrast to the behaviour of manganese and the alkaloid senecionine which while inducing slighter lesions in the liver tends to produce follicle necrosis and "fibro-adenie" in the spleen. It seems reasonable to assume that varying

toxins/

toxins may explain the difference in the morbid picture in man.

The Mode of Action and the Nature of the Toxic Factor.

It has been argued that splenomegaly of this type is at least in part the result of a direct toxic effect on the spleen quite apart from the vascular factor. Experimental work on the splenic reaction to cirrhogenic toxins has shown that the hepatic poison produces a simultaneous toxic effect on the spleen. McNee (1932) in his extensive studies on the problem has put forward a hypothesis of an alimentary metabolic tide from a short circuiting of the portal flow into the general circulation. McNee argues that the vascular mechanism is altered in cirrhosis so that the portal blood is diverted to collateral channels; the detoxicating effect of the liver is thus lost and toxic substances absorbed can reach the spleen through the general circulation. It seems however unnecessary to assume a distortion of the portal flow for inducing a toxaemia. Any hepatic disorder that can interfere with its detoxicating function can induce such a metabolic toxaemia. McMichael (1934) has demonstrated that hepatic involvement is a frequent feature of splenic anaemia. Clinical evidence in support of this view is the frequent association of splenic anaemia/

anaemia with attacks of jaundice, of chills and fever. It seems therefore reasonable to assume that a metabolic or other toxic factor, that can cause a hepatic disturbance and interfere with the "fixing effect" of the liver, can enter the general circulation and reach the spleen.

With regard to the nature of this toxic factor it is not possible to be more definite at this stage. In the absence of any known infective agent a toxin absorbed from the intestine is naturally suspect. It has also been argued that one which can produce hepatic damage and at the same time induce proliferative reactions in the spleen is probably involved. Experimental studies with cirrhogenic toxins have shown that these can induce varying grades of splenic damage and cellular proliferation. The studies of Rich (1935) have brought out the possibility that foreign proteins in the blood can induce cellular reaction in the spleen more or less similar to the acute splenitis of bacterial infections. Louros (1928) in a study of reticulo-endothelial reactions induced by carbohydrates, lipoids, proteins, metals, metallic salts and colloidal carbon has found that spleen lipoid and alkaline spleen extract the most active while oleokoniol, novoprotein, klaviprotein, managanese/

managanese chloride and colloidal carbon exhibit a descending grade of activity. The question of toxic agents which can induce varying grades of damage to the spleen cells and liberate splenic lipoid or splenic protein is therefore full of possibilities. Further work is needed however to settle this problem.

SUMMARY.

A histopathological study of splenic enlargement in 76 cases of liver cirrhosis, splenic anaemia and associated conditions, and 4 cases of Bengal splenomegaly have brought out the following facts:

1. Many of these cases show significant chronic inflammatory reactions in the pulp. In cirrhosis and in the later stages of splenic anaemia these are to some extent masked by venous stasis.

2. The congestive reaction is more common in the cirrhosis group, but is sometimes met with in the later stages of splenic anaemia of the Banti type.

3. In splenic anaemia and in splenomegalic cirrhosis, there is a marked reticulo-endothelial reaction to the inflammatory process, affecting the pulp cords as a whole or sometimes the littoral cells.

4. These reticulo-endothelial reactions are proliferative hyperplasias probably due to the inflammatory stimulus and are not real hypertrophies of the spleen.

5. In the later stages of splenic inflammation there is an associated fibrillary increase which is the splenic prototype of an inflammatory fibrosis in other organs.

6. The Banti lesion of "fibro-adenie" of the follicle is regarded as the result of a chronic folliculitis or/

or follicle necrosis due to a toxic spread from the vessels; this is from the analogy of experimental work (see Chapter II, Part I).

7. The condition called Bengal splenomegaly shows similar histologic reactions with, in addition, a marked accumulation of giant erythrophagocytes in the spleen.

8. A study of the splenic vein in 15 cases of splenic anaemia has shown only a patchy intimal thickening as a common lesion. In no case was thrombosis met with. There is therefore no support to the view that is put forward that the splenic anaemia syndrome is the result of a thrombophlebitis of the spleno-portal venous system.

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PATHOLOGICAL STUDIES ON SPLENOMEGALY.

PART II. SPLENOMEGALY IN MAN.

CHAPTER II.

THE SPLENIC REACTION IN KALA-AZAR.

THE SPLENIC REACTION IN KALA-AZAR.

INTRODUCTION.

As early as 1882 the prevalence of a type of tropical splenomegaly occurring endemically in certain parts of India (Garo Hills, Assam) had attracted attention. The disease was called "Kala-Jhwar" (Sanskrit "Black fever" or "Death fever"). It was first regarded as a postanaemic splenomegaly following anchylostomiasis (Giles, 1890) and later as a special type of virulent malaria (Rodgers, 1897).

Leishman (1903) first observed the causative organism in a soldier who had contracted the disease at Dum-Dum. He regarded the parasites as involution forms of a trypanosome. Meanwhile, Donovan (1903) in Madras obtained the organism both at autopsy and by puncture of the spleen during life. He held that the organisms were quite distinct from trypanosomes. Laveran and Mensil (1903) who studied Donovan's specimens suggested that the parasites belonged to the genus "Piroplasma". It is of interest that autopsy records of the Madras General Hospital about this period bear a diagnosis of "Piroplasmosis". However, Ross (1903) allocated the organisms to a separate genus "Leishmania" and they were subsequently known as Leishmania-donovani (Laveran and Mensil, 1903).

In/

In his classical histopathological studies Christophers (1904) demonstrated the affinity of the parasite for endothelial tissues of the spleen and the liver where they underwent intra-cellular multiplica-:tion followed by rupture of the infected cell and infection of neighbouring cells. These observations were confirmed by Statham (1905). He pointed out that the parasites were always intracellular in the endothelial and reticulum cells of infected organs and that the mononuclear cells and occasionally the polynuclear leucocytes were invaded.

Further pathological studies of Nicolle (1909), Visinteni (1910), Lombordo (1913), Dionisi (1913), Jemma and Di Christina (1914) and others pointed to the similarity of the lesions of the infantile type of the disease of the Mediterranean coast, to those of Indian Kala-azar, while Nicolle and Laveran (1913) established that the parasite Leishmania infantum (Nicolle, 1908) and Leishmania donovani (Laveran and Mensil, 1903) were identical.

Knowledge has also been gained regarding the extent and the distribution of the pathological lesions by the experimental transmission of kala-azar to dogs, rats, cats, mice, guineapigs and monkeys, both in Europe and in India. In most cases a transient leishmaniasis resulted. More recently, in China,

Young/

Young, Smyly and Brown (1924) have demonstrated the extreme susceptibility of the hamster as an experi-:mental animal and Meleny (1925) and Cash and Hu (1927) have shown that in infected hamsters the disease is essentially a parasitic invasion of the clasmatocyte system of cells in the body and that the spleen, the liver, the bone marrow, the lymphoid tissues, the intestinal sub-mucosa, the testis and even the sub-:cutaneous tissues are involved.

MATERIAL AND METHODS OF STUDY.

The material for study was obtained from 10 museum specimens in Madras. The time of autopsy varied from 18-24 hours after death. The specimens were all fixed in formol and mounted in glycerine and water, and so, a good deal of difficulty was met with in obtaining sections suitable for demonstration of Leishmania. Stained smears were also available in most cases. For sections, thin slices of tissue about 3-4 mm. in thickness were washed in running water for 24 hours to get rid of the glycerine and formalin, and were then post-fixed in Helly's fluid and sections obtained after paraffin embedding. In a few cases, frozen sections were stained and studied for the presence of fat and haemosiderin. The material in nine of these cases was from cases of kala-azar where an exact diagnosis was made after examination of spleen smears. In one case with a co-existing cirrhosis of the liver, the parasites were accidentally discovered in sections.

The staining methods used were as follows: (1) Haematoxylin (Mayer's haemalum) and eosine; (2) Turnbull's modification of Jenner's stain for tissues; (3) Giemsa's stain for tissues as modified by Wolbach; (4) Wilder's modification of Foot's stain; (5) Foot-Menard stain in a few cases; (6) Perl's Berlin blue reaction/

reaction for haemosiderin; (7) Sudan III for fat; (8) Iron haematoxylin stain in a few cases; (9) A modification of Leishman's stain for tissues as described in the appendix.

It was found that the Romanowsky stains were best for the demonstration of the parasites, expecially if the staining was prolonged. The parasites could also be demonstrated by the Foot-Wilder stain by overstaining with silver diaminohydroxide. This showed up the extent of the distribution of the para-:site though structural details could not be brought out. A formol precipitate was present in many speci-:mens but the histological detail could be well studied in spite of this.

Case I.

Spleen from a case of kala-azar.

Clinical notes. Patient A. aet 36. Male. Admitted for irregular fever, splenomegaly and cancrum oris; died 20-7-32.
(Government Royapuram Hospital).

Autopsy notes.(abstract) P.M. 187 of 20-7-32. Extensive cancrum oris involving the right tonsil and pharynx; enlarged cervical glands; slight dilatation of the heart; oedema and hypostatic congestion of lungs; enlarged fatty liver; enlarged spleen.

Spleen/

Spleen. Morphology Wt. 31 ozs (880 g) size much enlarged, capsule thin, tense, pinkish grey colour; cut surface bulging, soft, creamy pink to violet colour; trabeculae indistinct; Malpighian bodies not visible; pulp easily scraped off; splenic vein showed no thickening. No siderotic nodules. Smear showed Leishmania.

Histology (abstract). Capsule 80μ thick; slight perisplenitis; increased collagen, oedema, poor staining of nuclei; no infiltration. Trabeculae not thickened; slightly wider apart; no increased branching; oedematous; slight endothelial proliferation of veins. Slight mononuclear infiltration. Malphigian bodies small; marginal zones diffuse; cells mostly small lymphocytes; reticulum not increased; arterioles normal. Sinuses compressed; no engorgement; littoral differentiation. Walls not thickened. Billroth cords swollen from histiocytic differentiation and foamy degeneration of cytoplasmic reticulum; proliferation slight; deposit of blood pigment in reticulum cells; no marked congestion of the pulp. Free cells, marked lymphoid reaction in sinus; circulating leucocytes few; mononuclear cells in sinus and in pulp were the commonest type; these show marked vacuolation of cytoplasm; erythroblasts very few; no immature leucocytes; erythrophagocytosis; plasma cells frequent.

Fibrillary/

Fibrillary reticulum showed no increase. Parasitisa-:tion shown up by the silver stain. Parasites appeared as black coccoid bodies owing to formol fixation; parasitisation widespread, but not so heavy as in previous specimens. Large mononuclear cells and cytoplasmic reticulum most invaded; less so, the littoral cells.

Case II.

Spleen from a case of kala-azar. (Death due to dysentery).

Clinical notes. Patient S. aet 30, Male.

Admitted 12-7-32 to the Royapuram Hospital, Madras, with a history of irregular fever of two months dura-:tion, and an enlarged spleen; both legs oedematous, with punched out ulcers on ankles; enlarged cervical glands; died 17-7-32.

Autopsy notes (abstract). P.M.186/18-7-32. Fatty infiltration and dilatation of the heart; enlarged congested liver; enlarged spleen showing kala-azar parasites in smear; small capillary haemorrhages in large intestine - terminal dysentery.

Spleen. Morphology. Weight 31 ozs (880 g) size about four times the normal; capsule tense, slightly thick-:ened, edges firm and rounded. On section, pulp soft and bulging and had a pinkish violet colour; friable and soft to the touch; Malpighian bodies appeared as pin points.

Histology/

Histology (abstract). Capsule 100 μ oedematous; slight increase of collagen; serosa absent in places; slight lymphocytic infiltration. Trabeculae slight oedema; trabecular infiltration with lymphocytes and mononuclears in the smaller branches; trabecular veins showed slight subendothelial lymphoid reaction. Malpighian bodies small, but numerous; marginal zones indistinct; lymphoid tissue diffuse; mostly small lymphocytes; plasma cells at the marginal zone; arteriole slightly dilated. Marginal reticulum cells more prominent and invaded by parasites; no "fibro-adenie". Sinuses compressed; littoral differentiation into mononuclear cells; erythrophagocytosis; no engorge-:ment. Billroth cords, proliferation of cytoplasmic reticulum; histiocytic differentiation; cytoplasmic fibrils swollen and pulp spaces narrowed; vacuolation of reticulum cells marked; nuclei large and often degenerate. Free cells, large mononuclear cells; lymphoid cells numerous in sinus and pulp; leucocytes scanty; eosinophiles rare; plasma cells numerous in pulp; erythrophagocytosis. Fibrillary reticulum showed no increase in sinus walls, in pulp mesh, in periarterial and peritrabecular zones. Parasites most marked in free histiocytes and branching reticulum cells, less in littoral cells.

Case/

Case III.

Spleen from a case of kala-azar: death due to lobar pneumonia.

Clinical notes. Patient U. Woman aet 30. Admitted to the Royapuram Hospital, Madras on 5-5-32 for irregular fever and cough; oedema of the legs; slight distension of the abdomen; enlarged tonsils; cervical glands; died 12-5-32.

Autopsy Notes (abstract). Lobar pneumonia of right base; non-inflammatory fluid in all serous sacs; cloudy swelling of heart muscle and of both kidneys; enlarged spleen; conception in utero. Smear positive.

Spleen morphology. P.M. 183/13-5-32. Wt. 18 ozs (509 g.) size enlarged with two notches on anterior edge; organ soft and flabby. Capsule tense, easily torn; greyish pink colour; cut surface almost diffluent; purplish violet colour. Capsule transparent showing pin points of trabecular attachment; section showed trabeculae and vessels all obscured by pulp; no thick-:ening of splenic vein.

Histology (abstract). Capsule 160-200μ ; old peri-:splenitis; increased collagen. Trabeculae thickened, fibrous, slight increased branching; trabecular arteries, atheroma and medial fibrosis. Malpighian bodies few, atrophied; cells mostly small lymphocytes; no germ centres; reticulum slightly hyperplastic; Leishmania/

Leishmania in reticulum cells; no "fibro-adenie"; arteriole showed slight swelling of endothelium and hyalinisation of intima. Sinuses compressed, not engorged; littoral differentiation into mononuclear cells; hyperplasia slight; lymphoid cells in lumen. Billroth cords swollen from histiocytic differentiation; histiocytes appear bladder like from foamy degeneration; Free cells, Mononuclear cells in sinuses and pulp; lymphoid cells; occasionally plasma cells; polymorphs few; necrosis of mononuclear cells from parasitic invasion. Fibrillary reticulum round the malpighian arteries slightly increased; no increase in peritra-:becular zones, in sinus walls or pulp mesh. Parasites. Invasion and intracellular multiplication most marked in cytoplasmic reticulum of pulp, mono-:nuclear cells; next in littoral endothelium and least in lymphoid and perivascular reticulum. Vacuolation and foamy degeneration of the invaded cells terminating in late stages from intracellular multiplication in karyolysis and necrosis of affected cells.

Case IV.

Spleen from a case of kala-azar.

Specimen from the museum of the Madras Medical College from a case of kala-azar. No clinical notes available. Specimen N.1307.

Autopsy notes (abstract). P.M.3223. Grey hepatisation of/

of the left lung; dilated flabby heart; enlarged con-:gested liver; enlarged spleen showing kala-azar parasites in smear; passive congestion of kidneys.

Spleen morphology. Wt. 51 ozs (1448 gm.) size 27 x 15 x 5 cm.; there were two well defined notches anteriorly; organ soft, capsule slate grey in colour, slightly opaque; no perisplenitis; section pinkish red, pulp soft and bulging, almost diffluent; trabeculae indistinct, malpighian bodies not visible; splenic vein not thickened.

Histology (abstract). Capsule 80-100μ in thickness; slight increase of collagen. Trabeculae slightly thickened, fibrosed; nuclei feebly stained; oedema; trabecular infiltration slight; peritrabecular collec-:tions of lymphocytes and plasma cells; trabecular veins engorged; lining endothelium showed a few parasites. Malpighian bodies normal in size; marginal zones not sharp; cells mostly small and large lympho-:cytes; lymphoid reticulum slightly increased but not collagenous around arterioles; no definite fibro-adenie; a few germ centres; parasites scanty in reticulum; lymphoblasts few; ellipsodis seen in section, Sinuses compressed; walls not thickened; littoral endothelium slightly swollen and projecting into the lumen; some sinuses congested. Billroth cords markedly swollen; cytoplasmic reticulum foamy degeneration; histiocytic differentiation/

differentiation slight; erythrophagocytosis; pulp haemosiderosis. Free cells. Mononuclear cells in sinus and pulp; lymphoid cells numerous; plasma cells numerous in pulp mesh; polymorphs scanty; megakaryo-:cytes rare; no immature leucocytes. Fibrillary reticulum increased in periarterial and peritrabecular zones; increased branching in pulp with slight thick-:ening of fibrils. Parasites are not well stained but appear as black coccoid bodies owing to formol fixation. Parasitisation most marked in histiocytes and pulp syncytium, next in littoral cells and least in lymph reticulum cells. Endothelia of arteries showed no parasites; a few in veins.

Case V.

Spleen from a case of kala-azar.

Spleen from the museum of the Stanley Medical School from an old case of kala-azar; no clinical notes available; spleen smear showed kala-azar parasites. Specimen N.1453.

Spleen morphology. The organ is enlarged, measured 16.5 x 10 x 5 cm. Capsule thin, slate grey in colour, slightly wrinkled at upper pole; cut surface had a fleshy diffluent appearance; consistence soft, colour purplish violet fading to grey in places; no spots of haemorrhage; cut surface convex, trabeculae indistinct, malpighian bodies not visible; splenic vein slight thickening.

Histology/

Histology (abstract). Capsule 80μ in thickness; oedema; wavy wrinkling is lost from distension. Trabeculae widely separated; not thickened; no increased branching; trabecular veins show subendo-:thelial lymphoid collections and endothelial prolifera-:tion to slight extent; a few parasites in lining cells; free mononuclears containing parasites in lumen; slight peritrabecular infiltration; trabecular oedema. Malpighian bodies small, atrophied; marginal zones not distinct, irregular; small lymphocytes more than large; eccentric arterioles showed slight thickening of adventitia. Sinuses, wider under the capsule; lining cells ovoid, showing slight differentiation; sinus reaction more marked in medulla where littoral differentiation is more marked. Billroth cords swollen; cytoplasmic reticulum showed proliferation; histiocytic differentiation marked; foamy degeneration of cells marked; cytoplasmic fibrils thickened; Free cells mostly large mononuclears in pulp and sinuses. Many mononuclears necrotic; many lymphoid cells; a few plasma cells; eosinophiles and polymorphs few; other cells not met with. Fibrillary reticulum showed slight periarterial increase round penicillar vessels. No marked increase in pulp or sinus walls. Parasites. Many of the mononuclear cells loaded with parasites. Similar cells in pulp are heavily invaded; invasion of cytoplasmic/

cytoplasmic reticulum marked; slight in littoral cells, in advential cells of vessels, and in endothelial cells of veins. The lymphoid reticulum cells show slight parasitisation. Splenic vein: slight subintimal thickening; splenic artery healthy.

Case VI.

Spleen from a case of kala-azar; death due to peritonitis.

Clinical notes. Patient Mrs. A.M. Admitted to the Madras General Hospital for irregular continuous fever. She had at one time a small patch of cancrum oris which was excised and did not recur. Death due to peritonitis, cause unknown. Specimen F.34.

Autopsy notes (abstract). P.M. 841 of 22-7-14. Peritoneal cavity filled with purulent fluid; spleen enlarged, appeared separated by adhesions, and showing infarct; heart and lungs showed no gross abnormality; cause of peritonitis obscure unless it was due to extension from necrotic area in spleen.

Spleen. Morphology. Wt. 48 ozs. (1365 gm.) very much enlarged in a downward direction. Capsule greyish in colour; tense and not thickened except over two white infarcts. Pulp faded to a brownish grey colour; trabeculae indistinct; malpighian bodies not visible; dark zones present round infarct; pulp soft; cut surface flat; splenic vein not thickened. Smear negative.

Histology/

Histology (abstract). Capsule thick, 160μ ; increased collagen; slight infiltration of deeper layers; Trabeculae widely separated; increased collagen; slight peritrabecular infiltration; trabecular veins dilated; slight subendothelial reaction; Malpighian bodies atrophied and few in number; marginal zones are demarkated in a few; many show "germ centres" with hyaline necrosis - "reaction centres". Lymphocytes large and small but few lymphoblasts; reticulum increased; eccentric arterioles dilated; slight periarterial fibrosis. Sinuses under capsule and deep down are dilated, walls thickened; the littoral cells plump and sometimes protruding; marked mono-:nuclear differentiation; Billroth cords swollen from oedema. Cytoplasmic nuclei elongated and fibroblastic in type, little congestion; slight differentiation into mononuclear cells; little infiltration with leucocytes. Fibrillary reticulum shows moderate increase in the pulp ends; slight increased branching; reticulum of the follicles also increased. Parasites are few in number and found in large cells, in the pulp and in the sinus. The inrection in the cytoplasmic reticulum has apparen-:tly died down to a great extent.

Case VII.

Spleen from a case of kala-azar with cirrhosis of the liver.

Clinical/

Clinical notes. Patient M. aet. 35. Male, admitted 11-5-14 to the Madras General Hospital for irregular fever and enlarged spleen; diagnosis Chronic Malaria. Died 21-5-14.

Autopsy notes (abstract). P.M. 830/21-5-14. Liver enlarged and cirrhotic; heart flabby; bronchopneumonia left lung; enlarged spleen (F.126).

Spleen. Morphology. Wt. 70 ozs. (2017 gm.) uniformly enlarged; adhesion to diaphragm and under surface of liver; capsule thickened in patches with white spots of opacity; firm, cuts with resistance; cut surface flat of a reddish brown colour; not pig-:mented; trabecular markings prominent; malpighian bodies not visible; no siderotic nodules; splenic vein at hilum slightly thickened and enlarged. Smear positive.

Histology (abstract). Capsule thickened 160μ ; increased collagen; oedema; Trabeculae somewhat thickened; increased branching; oedema with loss of nuclear staining; lymphoid infiltration; slight peritrabecular infiltration; trabecular veins packed with mononuclear cells with parasites; slight endo-:thelial hyperplasia; trabecular splitting.

Malpighian bodies small, lymphoid tissue diffuse; marginal zones indistinct; cells mostly small lympho-:cytes; eosinophiles in the follicle; eccentric arterioles show marked thickening and fibrosis of media./

media. Sinuses distended and engorged under capsule; littoral cells rounded and protruding; sinus proliferation; mononuclear differentiation marked; erythrophagocytosis; thickening of sinus walls. Billroth cords compressed and thinned; sinus differentiation of the cytoplasmic reticulum; histiocytic differentiation. Free cells in sinus, lymphoid cells and mononuclears numerous; lymphoid cells, eosinophiles and histiocytes in pulp mesh; polymorphs scanty; red blood cells numerous in pulp. Erythrophagocytosis. Fibrillary reticulum markedly increased in sinus walls and in pulp mesh; fibrils thicker; increased branching; collagenisation of fibrils in pulp under capsule; "fibro-adenie" of pulp; slight periarterial increase. Parasites as black coccoid bodies; parasitisation more marked in cytoplasmic reticulum than in mononuclear cells; least in littoral cells. Parasites show small ring forms and torpedo forms from the effect of fixative; mostly intracellular.

Case VIII.

Spleen from a case of kala-azar.

Clinical notes. Patient C. aet. 35, male, admitted to the Madras General Hospital for dysentery and splenomegaly. The spleen had been enlarged for a month, occasional fever and rigor. Examination of motion showed a cellular exudate suggestive of bacillary/

bacillary dysentery.

Autopsy notes. P.M. 297/of 27-2-32.

General emaciation; lobar pneumonia of right lung; chronic ulcerative colitis; cloudy swelling of liver and kidneys; marked enlargement of the spleen.

Spleen. Morphology. Wt. 23 ozs. (610 gm.) much enlarged; capsule thickened, pulp soft, almost diffluent, deeply congested, malpighian bodies and trabeculae indistinct.

Histology. P.2594. Capsule 80μ ; oedema; slight infiltration; serosa peeling off; slight wavy wrinkling; Trabeculae oedematous, widely separated, slightly hyaline; peritrabecular infiltration; trabecular veins, subendothelial lymphoid reaction. Malpighian bodies normal in size, lymphoid tissue diffuse with loose reticular mesh; more small lymphocytes than large; endothelial lining of artery showed scanty parasites; arterioles dilated. Sinuses distinct; littoral differentiation; slight engorgement; no thickening of walls; haemosiderin deposit; Billroth cords, slight engorgement; loose mesh work; swelling of reticulum cells and thickening of cytoplasmic fibrils; histiocytic differentiation; hyperplasia of cytoplasmic reticulum; widening of cords from increase in tissue and cell accumulation; Free cells many lymphocytes and mononuclears in sinuses and in pulp; few polymorphs; immature cells rare; nucleated red cells rare; most of mononuclear/

mononuclear cells swollen with foamy degeneration; some necrotic; Parasites, invasion of cytoplasmic reticulum extensive almost invariable; parasites well stained appearing as pale blue cocci inside vacuoles in affected cell; black irregular granules also met with not due to any pigment but from formol fixation; cytoplasmic threads also show invasion; marked invasion of mononuclear cells with degeneration of infected cell; invasion of lymphoid reticulum cells; littoral cells also invaded but not so markedly as the cytoplasmic reticulum; invasion of endothelia of vessels of veins; polymorphs occasionally show parasites.

Case IX.

Spleen from a case of kala-azar.

Clinical notes. Patient K. aet. 30, male, previously admitted and treated in Government Royapuram Hospital for Kala-azar; readmitted for dysentery. Spleen enlarged to the umbilicus; liver palpable.

Autopsy notes (abstract). P.M. 222 of 1-8-33. General emaciation; cloudy swelling of the heart muscle and kidneys; hyperstatic congestion of both lungs; enlarged fatty liver showing Leishmania in smear; enlarged spleen showing numerous Leishmania in smear; amoebic ulceration of the large intestine.

Spleen. Morphology. Wt. 26 ozs (712 gm.) much enlarged/

enlarged; capsule slightly thickened; slight peri-:splenitis; in section soft, bulging, convex, cut surface of a violet colour; trabeculae indistinct. Histology (abstract). Capsule 80-100 μ, oedematous and necrotic in places; slight fibrosis. Trabeculae not thickened, separated and softened with poor nuclear staining; slight subendothelial lymphoid reaction of trabecular veins; numerous mononuclears free inside veins; Malpighian bodies somewhat atro-:phied; central hyaline necrosis of reticulum cells; marginal zones indistinct; small lymphocytes more numerous; lymphoid reticulum not increased. Eccentric arteries show swelling of lining endothelium; Sinuses well defined, walls swollen; little engorgement; marked littoral differentiation; no sinus prolifera-:tion; slight erythrophagocytosis; irregular proto-:plasmic bridges projecting from sinus walls and cell debris inside sinus suggest disintegration of littoral cells and free monocytes; littoral cells show slight vacuolation from parasitic invasion; cells plump ovoid or rounded. Billroth cords swollen from cell accumulation and histiocyte differentiation; cytoplasmic reticulum swollen and irregular with thick cytoplasmic fibrils; degenerative softening of the cell with karyolysis common; moderate congestion of the pulp mesh. Free cells, besides mononuclear cells lympho-:cytes/

:cytes most numerous in sinuses and pulp; a few plasma cells and polymorphs. Mononuclear cells show eccen-:tric displacement of nucleus pyknosis and karayolysis and foamy degeneration of cytoplasm. Parasites appear as black rings or ovoids inside the invaded cells. Parasitisation is most marked in the cyto-:plasmic reticulum and the mononuclear cells and histiocytes; the littoral cells are also extensively invaded; the lymphoid reticulum is least affected; endothelia of veins also show parasites while those of arterioles are not involved; intracellular multi-:plication results in cells with 40-50 parasites causing extreme enlargement vacuolation and formation of "bladder cells" with eccentric pale staining nuclei.

Case X.

Spleen from a case of kala-azar; old malarial infection.

Clinical notes. Patient M. aet. 35, admitted to the Madras General Hospital on 11-5-14 for irregular fever and an enlarged spleen. Diagnosis: chronic malaria.

Autopsy notes. P.M.830 of 21-5-14. Liver enlarged ? cirrhosis; no free fluid in the serous cavities; patch of bronchopneumonia in left lung; spleen very much enlarged.

Spleen. Morphology. Specimen F.13. Wt. 70 ozs (1985 gm.) uniformly enlarged; adhesions to diaphragm and/

and liver; capsule thick, opaque irregular white spots; cut surface reddish brown; trabecular marking increased; consistence firm.

Histology (abstract). Capsule 280μ, marked increase of collagen; old perisplenitis. Trabeculae: oedema and softening necrosis; slight infiltration; malarial pigment in clumps. Malpighian bodies: atrophied; more small lymphocytes than large; no increase in reticulum; malarial pigment in cells; eccentric arteries slight periarterial fibrosis; marginal zones thickened. Sinuses well demarkated, slightly dilated; littoral differentiation. Billroth cords swollen from enlargement of reticulum cells and slight hyperplasia; clumps of malarial pigment in cells; slight histiocytic differentiation; small haemorrhage under capsule. Free cells: lymphocytes, mononuclear cells and histiocytes; many plasma cells; few polymorphs. Fibrillary reticulum: slight increased branching and thickening in pulp; periar-:terial increase. Parasites; dense clusters of malarial pigment in reticulum cells and in the trabeculae indicate old infection. Leishmania: most in the pulp cells and less in the littoral cells; infection is not marked as in other specimens; lymphoid reticulum shows parasites. Splenic vein: no changes. Splenic artery: no changes.

COMMENT.

The splenomegaly of kala-azar is generally the most noticeable feature in the morphology of the disease. The enlargement is generally extreme, even greater than in malaria, with the spleen sometimes extending down even to the pelvis. In this series of cases, the weight varied from 18-70 ozs (609 - 2016 gm.). In an analysis of autopsy records in South India the maximum enlargement recorded in kala-azar was 80 ozs (2265 gm.), (Menon, 1934). Marchand (1927) records a maximum of 1710 gm. Meleny's figures (Meleny, 1925) varied from 485 - 1355 gm. Peri-:splenitis was not a common feature, unlike what obtains in chronic malaria. In two cases recorded in this series, it was due to complications. Gross capsular thickening and fibrosis were not common. On the other hand, the capsule was generally thin and stretched, and usually transparent showing the pulp shining through. Bramachari (1928) mentions infarcts as common, but they were met with only in one of this series. In infantile kala-azar, Giraud and Pursines (1934) found none in thirteen cases. The shape was generally well preserved, but on section, the pulp was more or less soft and bulging so that the trabeculae and malpighian bodies were obscured. The swelling of the/

the pulp was so marked that in some cases a puncture through the capsule resulted in the herniation of a small bead of pulp. A tear could easily be made emphasising the danger of movement during the opera-:tion of diagnostic spleen puncture. The pulp was of a reddish violet colour when fresh, but had faded to a dark grey after fixation. It was very friable, and in fixed specimens had a granular appearance. In some cases, the pulp was frankly diffluent. A firm consistence was met with only in two cases which were probably chronic. Exceptionally, the malpighian bodies appeared as greyish pin points. The splenic vessels at the hilum showed no gross changes except slight enlargement and widening of the lumen. Phlebitis was not met with.

Histologically the thickness of the capsule varied from 80-100μ. Marked thickening was met with only in two cases. The collagen fibrils appeared swollen from oedema and showed poor nuclear staining. The elastic fibrils were thin and stretched. Occa-:sionally a few lymphocytes and mononuclear cells had wandered into the capsular tissue. The changes in the trabeculae were similar. Softening from oedema and poor nuclear staining were common. There was little trabecular splitting and spread of collagen fibrils into the pulp. Peritrabecular infiltrations were/

were little marked. The smaller branches showed slight lymphocytic infiltration. A sub-endothelial lymphoid reaction in the trabecular veins was much less marked than in malaria. Parasitic invasion of the lining endothelium was met with only in two cases. There was no dilatation to suggest venous stasis. The trabecular arteries were more or less normal. As a rule, the changes in the capsule-trabecular system were degenerative and suggested toxic softening. Fibrosis and thickening were exceptional.

The characteristic histopathological changes could be grouped as follows:- I. The atrophy of the malpighian follicles; II. The sinus reaction; III. The enlargement of the pulp cords; IV. The parasitic invasion.

I. The atrophy of the lymph follicles was a well defined feature in most of these cases. Signs of activity of the follicles were not met with. The cells were mostly small lymphocytes with a variable proportion of large lymphocytes, while large lympho-:blasts were almost absent. A toxic type of follicle reaction with necrosis of the central reticulum cells was met with only in one case where death was due to peritonitis. Appearances suggestive of "fibro-adenie" occured in one case with a complicating cirrhosis. Giraud and Poursines(1934) and Franco (1922) have described/

described such changes commencing around the arterioles and at the marginal zones in infantile kala-azar, but it is uncertain whether these changes merely represent an enlargement of the reticulum cells or a fibrillary increase. In the present series, an enlargement and increase of the lymphoid reticulum cells were met with in four cases; but this was due to parasitic invasion which had also affected the adventitial cells of the arterioles and penicilli. The marginal zones of the follicle were irregular and the cytoplasmic reticulum swollen with parasites indicating that the atrophy of the follicle was more related to an encroachment by the hyperplastic pulp. The endothelium of the arterioles was free from parasites. De (1934) has emphasised this feature, though the earlier workers (Dionisi, 1913 and Schilling, 1925) have reported that these cells are primarily involved. It is however the swollen reticulum cells round the vessels that are occasionally invaded, and not the lining endothelium.

II. The sinus reaction was characterised by moderate littoral hyperplasia, by the swelling of the littoral cells and by differentiation into mononuclear cells. The typical feathery appearance of advanced littoral hyperplasia was only sometimes met with, but swelling and shedding of cells and fragments of dis-:integrating cells gave rise to picture somewhat like the/

the renal tubules in nephritis. Parasitic invasion of the lining cells and mononuclear leucocytes was only less marked than in the pulp. The mononuclear cells varied in size, some were not parasitised, while others showed a varying extent of intracellular multiplication, to form giant mononuclear cells 30-40 μ in size. Karyolysis and complete disappearance of the nuclei were sometimes met with, and some cells appeared disintegrating from extreme intracellular multiplica-:tion. Erythrophagocytosis was present. A notice-:able feature was the presence of numerous lymphocytes within the sinus while plasma cells were also frequent. Engorgement was not marked.

III. The widening of the pulp cords was in part due to parasitic invasion of the cytoplasmic reticulum, in part due to differentiation into free amoeboid cells and also to the accumulation of cells within the mesh. The reticulum cells were much enlarged, irregularly quadrangular and loaded with parasites. The cyto-:plasmic fibrils were swollen and the mesh spaces reduced in consequence. Owing to the hyperplasia of the pulp the cords appeared as solid sheets with a scanty mesh. The nuclei of the cells were large, pale and ovoid and had often lost the convoluted appearance of the nuclear membrane while the cytoplasm had a foamy appearance from the presence of numerous vacuole/

vacuole like spaces surrounding multiplying Leishmania. Frozen sections stained with Sudan III showed little fat or lipoid material. Haemosiderin deposit was slight and more marked round blood vessels and trabeculae. Extensive parasitisation of the mono-:nuclear cells was associated with necrosis. Free Leishmania could be demonstrated in small numbers round disintegrating cells. The engorgement of the mesh was variable, but never well marked. A note-:worthy feature was the presence of focal collections of plasma cells within the pulp. Hu (1933) has drawn attention to this reaction in the spleens of hamsters infected with Leishmania. Polymorphonuclear leucocytes were few. Lubarsch (1927) has described multinucleated giant cells of the Langhan's type, but these were not met with in any of this series. Erythroblasts were occasionally met with and were very numerous in one case where the spleen was extensively parasitised. Myelocytes were sometimes present, but in very small numbers depending on the degree of reactive changes in the bone marrow, since this is also parasitised. An increase in the fibrillary reticulum of the pulp was found only in two cases. Giraud and Poursines (1934) regard this as frequent in the infantile type of the disease. On the other hand Shanks and De (1931) found reticular increase only/

only in 20 per cent of their cases of Indian kala-azar. The reticular increase would appear to vary with the chronic forms of the disease, since in the early stages the cellular hyperplasia would mask any slight fibril formation.

IV. The appearance of the parasites depended to a very great extent on the freshness of the tissues, the fixative and the staining methods used for their demonstration. It was noticed that if the autopsy was performed more than twenty-four hours after death, the chromatin appeared disintegrated and the parasites unstained. With formol fixation the Leishmania appeared as larger oval, rounded, or irregular rings or coccoid bodies where the chromatin could not be distinguished. Often the more superficially placed parasites appeared black while the deeper ones showed nuclear staining. That this was a definite fixative effect could be demonstrated by comparison with fresh stained smears where the black coccoid appearance was not seen, but the parasites appeared well stained. With regard to the morphology of the parasites, they appeared in sections much smaller than in smears owing to shrinkage caused by the fixative. The cytoplasm and the parabasal body were only feebly stained. Intracellular multiplication was by binary fission, though/

though sometimes in smears owing to rapid nuclear division forms were met with where the cytoplasm had not completely separated, an appearance which has been mistaken for schizogony. Free parasites were very few and found only around ruptured cells, suggesting that the cycle of development in the body was intra-:cellular. In case X the effect of an old malarial infection in interfering with the activity of the pulp cells was shown by the relatively few Leishmania that had invaded the cells of the pulp and the free histiocytes.

Fig.I. Case II (x 110) showing the slight fibrillary increase of the capsule and the cellularity of the pulp. (Haemalum and Eosine).

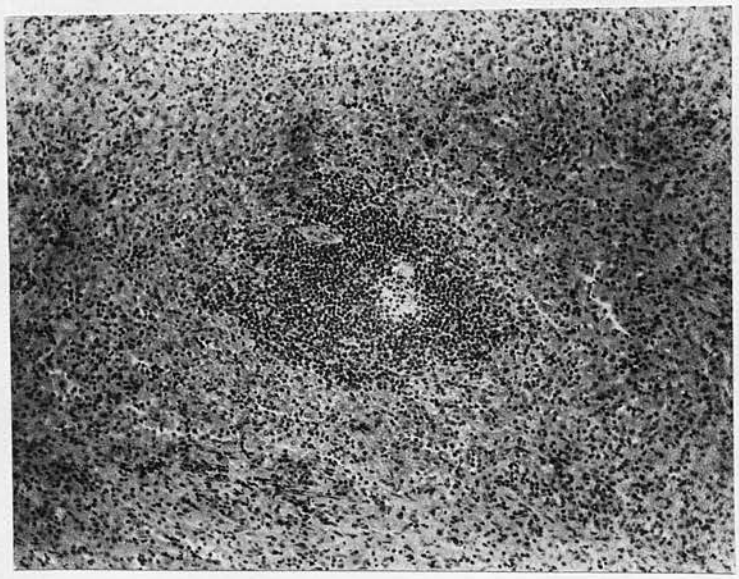

Fig.II. Case II (x 110) showing the atrophy of a malpighian follicle with a slightly hyaline node in the centre; note the absence of any fibro-adenie. (Haemalum and Eosine).

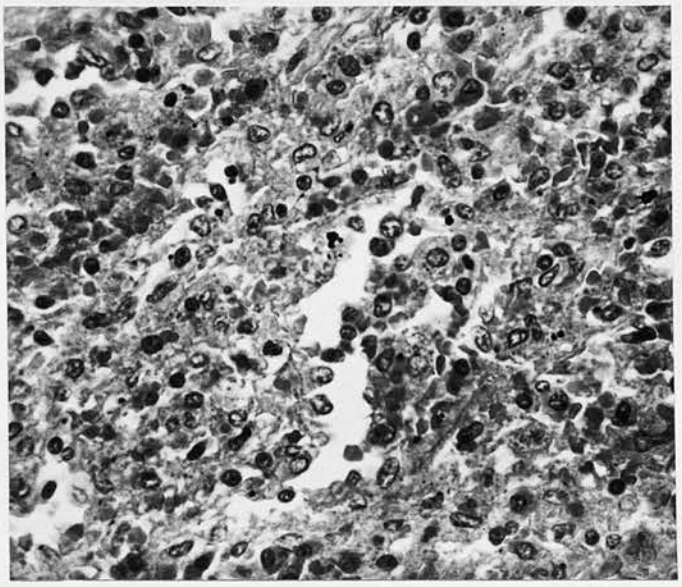

Fig.III. Case II (x 500) showing the cytoplasmic increase of the pulp, the swelling of the pulp mesh and the sinus reaction with swelling of the littoral cells and differentiation.

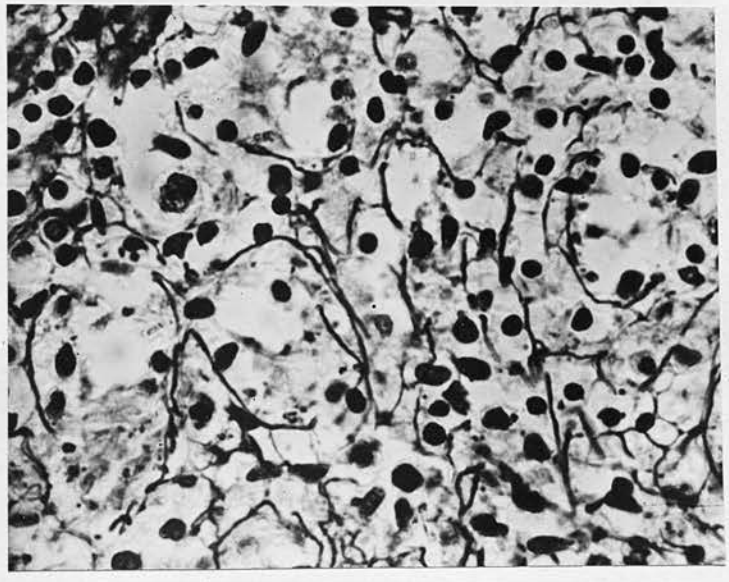

Fig.IV. Case (x 700) showing the loose reticular mesh without any marked thickening or increased branching. Foot-Wilder stain.

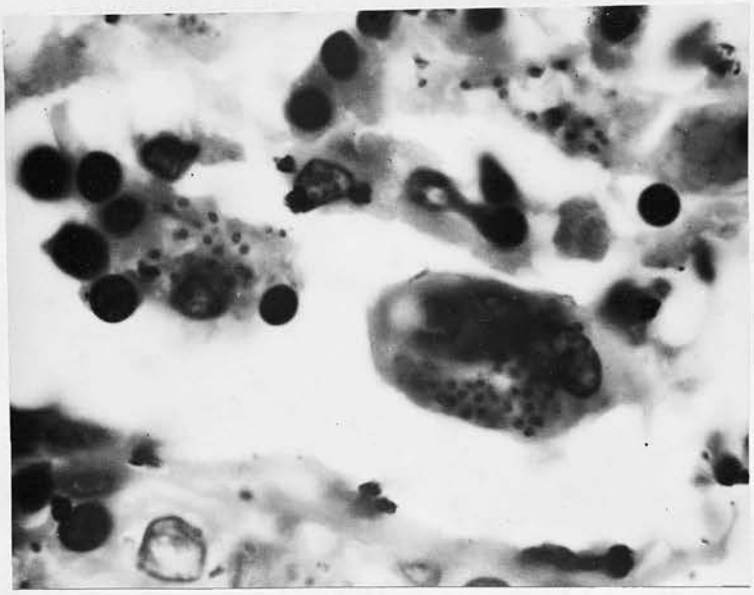

Fig.V. Case VIII (x 1500) showing two large mononuclear cells heavily invaded within the sinus while the littoral cells show only slight invasion. (Haematoxylin and Eosine).

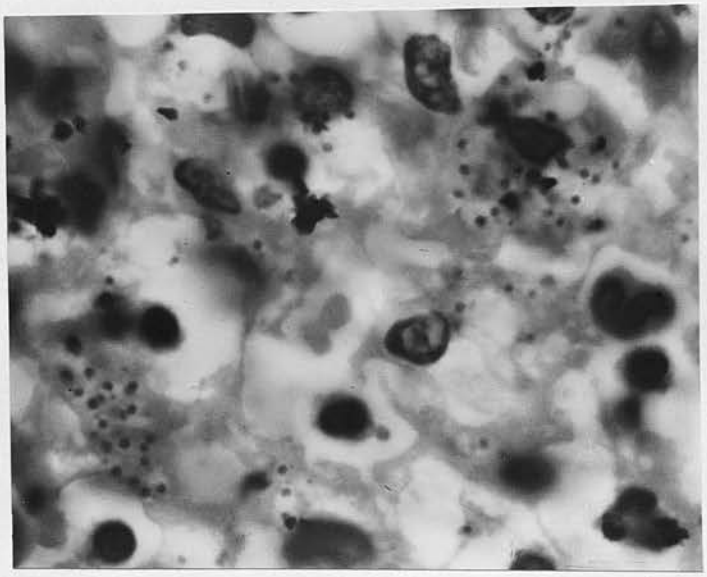

Fig.VI. Case VIII (x 1500) showing the parasitic invasion of the cytoplasmic reticulum, the parasites looking like cocci surrounded by faintly stained cytoplasm. (Haematoxylin and Eosine).

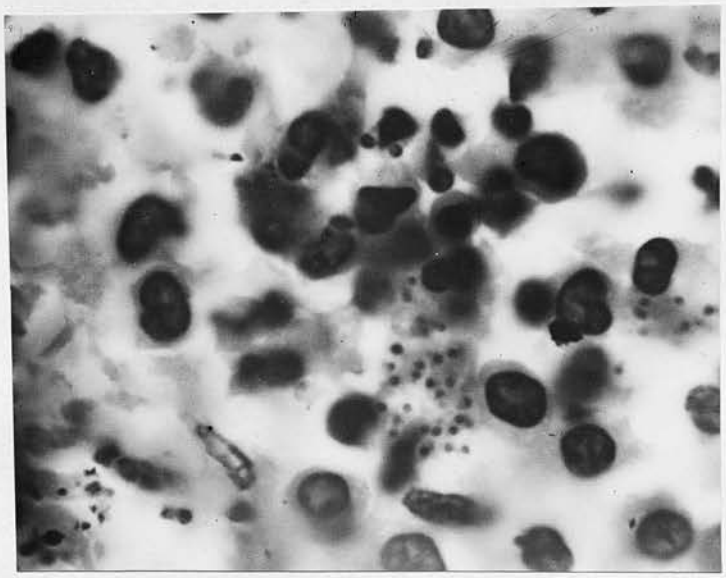

Fig.VII. Case VIII (x 1500) showing a lymph follicle with invasion of the reticulum cells of the follicle by Leishmania. (Giemsa's stain).

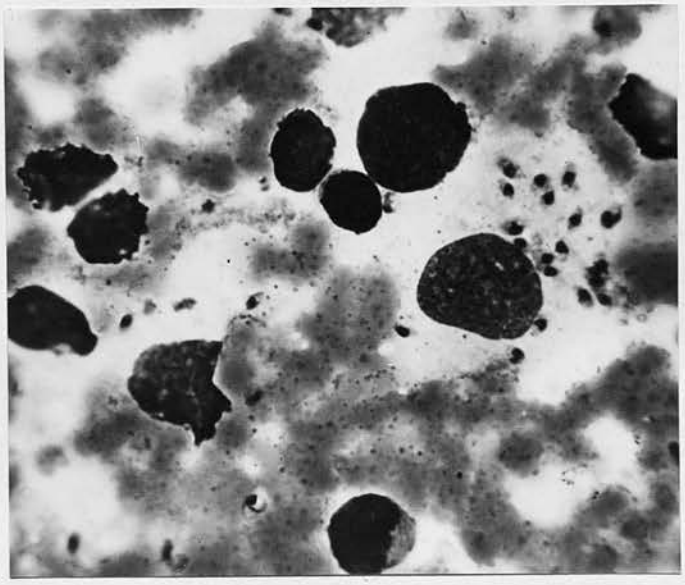

Fig.VIII. (x 1500) showing the typical appearance of Leishmania around a ruptured large mononuclear cell (spleen smear). Note the round chromatin of the nucleus, the rod shaped parabasal body and the ovoid shape of the parasite. (Leishman's stain).

DISCUSSION.

Kala-azar as a reticulo-endothelioses.

The extent and the distribution of the parasites in the spleen offer a striking parallel to the distri-:bution of dyes such as isamine blue, trypan blue or lithium carmine when they are injected intravenously into the circulation. The Leishmanial infection and intracellular multiplication are most marked in the reticulum cells of the pulp and the large mono-:nuclear cells derived therefrom, in other words, the fixed histiocytes and the free histiocytes of the spleen. The mononuclear cells within the sinuses show a varying extent of parasitic invasion probably associated with the varying size and activity of the cell. Occasionally many cells are found which are not parasitised at all, while giant mononuclear cells appear which are loaded with Leishmania. A careful study of the distribution of the parasite within the littoral cells has demonstrated that these cells are invaded to a lesser degree than the reticulum cells of the pulp, though Aschoff (1924) in his classifica-:tion regards the latter cells as less active than the endothelial cells of the sinuses. In blood films in cases of kala-azar the large mononuclear cells (the true monocytes so called), show parasites in very small numbers as compared with the large phagocytic cells/

cells of the spleen. Krishnan, Lal and Napier (1933) in their haematological studies claim that, by supra-:vital staining, these cells can be distinguished in the blood in kala-azar, and that they appear in vary-:ing proportions in the beginning and at the end of infection. Whatever be the histiogenesis of these cells, there seems to be some support to the view of Cunningham, Sabin and Doan (1924) that there exist in the spleen pulp two types of phagocytic cells which vary in the degree of their functional activity. That the adventitial cells of the vessels are also invaded is only in keeping with the known phagocytic activity of these cells as first demonstrated by Marchand (1890). The gradual involvement of the lymphatic reticulum cells of the malpighian bodies is of some significance in view of the widespread lymphatic involvement that has been recently des-:cribed in the disease. The parasites have been found in the tonsil (Forkner and Zia, 1934), in the adenoid tissue of the naso-pharyngeal region (Grilli, 1935), in the nasal secretion (Forkner and Zia, 1935; Shortt and Swaminath, 1935), in the lymphatic glands (Giraud and Caudière, 1926; Napier and Muir, 1923) and occasionally in the thymus (Thompson and Robertson, 1929). The massive localisation in the spleen, the liver and the bone marrow is only proportional to the amount of reticulo-endothelium that is capable of being/

being parasitised. Evidence of the widespread nature of the infection is shown by the invasion of the ramifications of the reticulo-endothelial system, as in the adrenal (Jemma and Di Christina, 1911; Meleny, 1925; De, 1934), in the testis (Shortt, 1923; Hindle and Thompson, 1928), and in the lung (De, 1934).

The presence of the parasite in the skin and gastro-intestinal tract has been variously explained. Christophers first described them in granulomatous masses in the intestinal mucosa, while Perry (1922) found large clusters of parasitised mononuclear cells in the villi. In infected hamsters, Meleny found parasites in clasmatocytes diffusely scattered throughout the submucosa while Cash and Hu (1927) found clusters of infected clasmatocytes in all layers below the epidermis. Meleny thus concludes that the disease is an infection of the cells of the clasmato-:cyte system of Ranvier (1891). On the other hand, De (1934) suggests that the occasional gastro-intesti-:nal and cutaneous localisations are secondary to some local inflammatory process which provokes a histiocyte response. The present study tends to show that the primitive reticulum cells are first affected, that differentiation into free histiocytes with marked powers of phagocytosis is a more gradual process, that the littoral cells are less involved than the primitive reticulum/

reticulum cells of the pulp, that it is the adventitial cells of the capillaries and smaller arterioles that are often invaded and not the lining endothelium, that in heavy infections the lymphatic reticulum cells are invaded, and that there is gradual spread of infection from the littoral endothelium to the lining cells of the pulp veins and occasionally the trabecular veins. It seems probable that the occasional infection of the intestinal submucosa, of the tonsil and of Waldeyer's ring in the naso-pharynx might be due to an activation of the lymphoid reticulum cells which are histiogeneti-:cally part of the histiocyte system as defined by Aschoff and Kiyono (1913).

Forkner and Zia (1934) have advanced the argument that the gastro-intestinal, pharyngeal and tonsillar localisation is evidence of a mode of infection through the alimentary canal. Such localisations are however, only occasional and not invariable. Hindle and Thompson (1928) have pointed out that intra-:peritoneal injections of Leishmania in hamsters are followed by a primary involvement of the mesenteric glands and a subsequent spread to the liver, spleen and bone-marrow with the parasites appearing in the blood. In human infections however, kala-azar shows no primary lymphangitis or a primary localisation in the lymphatic glands. When lymphatic involvement is present/

present, the spleen is generally enlarged and parasites are found in the gland only in small numbers.

Toxic Reactions in Kala-azar.

Evidence of toxic reactions in spleen is usually met with. The changes within the sinus have been regarded as inflammatory (Giraud and Poursines, [illegible]. (1934), but the appearances suggest differentiation rather than a toxic or inflammatory catarrh with desquamation. In the pulp cells however one sees feeble nuclear staining and a picture suggestive of necrosis from extreme parasitic invasion. In fresh smears from the spleen pulp from recent autopsy material some of the intact cells invaded with Leishmania show little cytoplasmic degeneration. It would seem that only extreme intracellular multiplication has an effect in causing necrosis and disintegration of the infected cell. Even then the morphology of the parasites is preserved. One finds a striking similarity to the behaviour of histiocytes which have ingested indigestible matter such as colloidal silver or indian ink. There is little intracellular destruction but the histiocytes degenerate, the particles are set free and are taken up by other histiocytes.

No evidence of an allergic type of reaction is present indicating that after rupture of distended cells the parasites are not destroyed in situ, but are soon/

soon engulfed by neighbouring cells. Destruction is probably intracellular if it takes place as a result of treatment.

The plasma cell reaction in the pulp is of importance if they indicate a reaction to an excess of serum protein filtered by the spleen. Müller (1932) argues that the plasma cell reaction in the spleen is due to an excess of protein in the blood as it is met with in caseating tuberculosis and malignant tumours undergoing necrosis. It is of interest that such an excess of serum protein has been demonstrated in the blood in kala-azar by Bramachari (1916), and also by Napier (1922) and Lloyd and Sen (1928) who pointed out that the increase is due to euglobulins.

The marked leucopenia of the disease, the fre-:quency of cancrum oris and the occasional agranulo-:cytosis as reported by Zia and Forkner (1934) are also features of interest. A toxic destruction of the neutrophile leucocytes, a possible leucocidin effect cannot be demonstrated in the spleen. In smears the leucocytes appear normal in shape. The leucopenia can be better correlated with a depression of myelopoiesis in the parasitised bone-marrow. This would also explain the relative lymphocytosis in the blood since the lymphoid tissues are not so extensively involved.

The anaemia of kala-azar has been regarded as due to/

to an increased erythrolysis in the haemopoietic organs and from a general increase in the blood and tissue histiocytes (Napier and Sharma, 1932; Kassirsky, 1934). However, haemosiderin though present in the spleen is not so marked as in haemolytic anaemias such as acholuric jaundice. It seems possible that the anaemia at least in part is due to a defective formation of the erythrocytes in the invaded bone-marrow.

The frequency of inflammatory complications with fatal effects lend support to the view that a reticulo-endothelial "blockage" is responsible for the failure of the defense of the organism against an infective process.

ACKNOWLEDGMENT.

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PATHOLOGICAL STUDIES ON SPLENOMEGALY.

PART II. SPLENOMEGALY IN MAN.

CHAPTER III.

THE PATHOGENESIS OF EGYPTIAN SPLENOMEGALY.

THE PATHOGENESIS OF EGYPTIAN SPLENOMEGALY.

INTRODUCTION.

From the evidence of the existence of bilharzial disease in ancient Egypt as shown by the researches of Ruffer (1910) into the pathological anatomy and histology of mummies, it seems probable that bilharzial splenomegaly was also prevalent. It was only recog-:nised however, in modern medicine after the report of Roger (1903) on two cases of splenectomy in a type of Banti's disease that was met with in Egypt. The association of this splenic enlargement with hepatic cirrhosis was also pointed out by Day and Ferguson (1909) and Ferguson in 1910 described the hepatic lesion as similar to the common coarse cirrhosis that was met with in Europe. The disease was for a time confused with malaria (Kartulis, 1912; Phillips, 1912). The haematological findings of Day (1911) and of Ferguson (1912-13) were however against this view. The analogy to some clinical types of infection with Schistosomum mansoni was pointed out by Manson Bahr (1921). Subsequent studies have established the bilharzial origin of this condition. Thus El Quadi (1923) demonstrated the ova of the parasite in the faeces in cases of splenomegaly while Day (1924) found them in pieces of the liver removed during the opera-:tion/

:tion of splenectomy. Coleman and Bateman (1924) had arrived at a similar conclusion regarding the origin of the condition. In further work, Coleman (1926) confirmed the finding of the lateral spined Sch.mansoni ova in the liver at operation. Meanwhile the dis-:covery of the siderotic nodule in cases of Egyptian splenomegaly as in Banti's disease in Europe had swung the pendulum in favour of a mycotic theory and Askanazy and Schweizer (1927), Schweizer (1927) and Petridis (1931) subdivided the disease into two types, one show-:ing the nodules which was mycotic and the other possibly bilharzial. With the demonstration of the real haemorr-:hagic nature of the nodule confirming the original views of Sprunt (1914) by a series of workers (Cristeller and Puskeppelies, 1924; Gamna, 1928; Langeron, 1928; Hueck, 1928; McMichael, 1931) the duality of the types became untenable.

Meanwhile the Japanese workers had succeeded in infecting cattle, cats and dogs with the allied Sch. japonicum which causes the "Katayama disease" of Japan where visceral localisation of the parasite is associated with a definite splenomegaly. Following Cobbold's discovery (Cobbold quoted by Fairley, 1920) of a natural schistosome infection in the African monkey Cercopithecus fuliginosus, the experimental studies of Fairley (1920) with monkeys infected with Sch./

Sch. mansoni and Sch. haematobium had brought to light the close similarity of the hepato-lienal syndromes in the experimental animal and the disease process in man.

Girges (1932) in a review of the aetiological factors has pointed out that the distribution of the disease along the river banks and canal zones, its predominance in males who form the working class population in Egypt and its special prevalence among the Egyptian "fellaheen" and "fulkies" (the peasants and priests) all favour the view of infection with Sch. mansoni, even quite apart from the demonstration of lateral spined ova in the tissues. Opinion however has not been agreed with regard to the mode of action of the parasite and especially in regard to the mechan-:ism of production of the splenomegaly and the hepatic lesion. With regard to the infecting species, Onsy (1937) implicates both the worms as he has been able to find their ova in a large number of cases. On the other hand, Day (1933) stresses the analogy of Sch. mansoni infection in man to the "katayama disease" of Japan with spleno-hepatic and intestinal localisation and argues that the old idea of a pure intestinal type of Sch. mansoni infection is probably erroneous.

Material and Methods.

Material for study was obtained from 4 cases which had come up for autopsy in Egypt and 3 cases where the spleens were removed at operation. The autopsy material was sent by Professor A.F. Bernard Shaw and the splenectomy specimens were sent by Dr. S.M. Aidaros from Egypt. One specimen was obtained from Dr. J. McMichael. In the first seven cases the material was obtained from different portions of the same spleen and in some the hilar vessels were included. Professor Bernard Shaw has also sent complete clinical and laboratory findings and detailed autopsy reports of his four cases. Only abstracts of these are included here. The tissue was fixed in Helly's fluid embedded in paraffin and sections stained by the following methods: (1) Mayer's haemalum and eosin; (2) Heidenhain's azan stain; (3) Anderson's iron haematoxylin and Van Gieson's stain; (4) Leishman's stain for the spleen as described in the appendix; (5) Wilder's modification of the Foot-Bielschowsky stain for reticulum; and (6) Perl's prussian blue reaction for the demonstration of haemosiderin. Various chemical tests were used for the study of bilharzial pigment; these are described elsewhere.

RESULTS.

Specimen No.	Record No.	Name	Age	Short clinical abstract.	Autopsy abstract.	Spleen and splenic portal veins.	Capsule	Trabeculae	Siderotic nodule	Peri-arterial fibrosis	Malpighian follicles	R.E. Proliferation.	Inflammatory reaction	Sinus proliferation	Congestion P = Pulp S = Sinus	Haemorrhage T = Trabecular P = Pulp	Bilharzial pigment	Remarks.
E.1	Specimen from a case of Egyptian splenomegaly after splenectomy; liver early cirrhosis; spleen enlarged wt. 500 gm.; from Dr. S.M. Aidaros.						Hyaline	Peri-trabecular infiltration	Nil	+	Variable atrophy	?	Plasma cells; Monocytes; Megakaryocytes.	?	P = + S = +	P = +	+	Fibrillary increase = ++
E.2	Specimen from a case of Egyptian splenomegaly after splenectomy; spleen wt. over 500 gm.; from Dr. S.M. Aidaros.						Hyaline thickened	Peri-trabecular infiltration	Nil	+	Atrophy	++ Littoral reaction	Eosinophiles +++ Monocytes.	+	Nil	Nil	+ Diffuse Granular	Fibrillary increase ++
E.3	Specimen from a case of Egyptian splenomegaly after splenectomy; spleen wt. over 1000 gm.; from Dr. S.M. Aidaros.						?	Trabeculitis	Nil	+	Hyperplasia	++ fibroblastic change. Littoral reaction.	Monocytic Eosinophile +++	++	Nil	Nil	+++ Granules & Blocks	"Fibro-adenie" +++
E.4	P.M.6 1937	G.H. el.A.	12	Dyspnoea, anasarca Bilharzia ova in urine; subacute nephritis.	Ascitis; coarse cirrhosis; patent foramen ovale; early broncho-pneumonia.	Spleen 310 gm. veins healthy; no worms or ova; no s.nodules.	Nil	Nil	Nil	+ Inflammation	No change	+ Littoral reaction	Monocytic Plasmacytic Lymphocytic	+	Nil	Nil	+ Granules Blocks	Fibrillary increase +
E.5	P.M.46 1937	A.H. el.A.	20	Acute laryngeal obstruction; splenomegaly.	Liver nodular; early cirrhosis; streptococcal angina.	Spleen 2000 gm. no s. nodules; portal vein healthy.	Thickening	Peri-trabecular infiltration	Nil	+	No change	+	Slight Monocytic Lymphocytic	+	P = +	T = +	+	Fibrillary increase ++ Collagenisation +
E.6	P.M.52 1937	B.H. M.	60	Dyspnoea; pyuria; history of haematuria; diagnosis carcinoma bladder.	Bilharzial carcinoma bladder metastasis; hydronephosis; splenomegaly early pipe-stem cirrhosis.	Spleen 850 gm. s. nodules; splenic and portal vein healthy; no worms.	Thickening	Thickening	++	+++	Atrophy	+	Slight Monocytic Lymphocytic	+	P = +	-	++ Granules & Clumps	Fibrillary increase ++ Artery hypertrophy
E.7.	P.M.54	A.M.		M.T. malaria; anaemia; cirrhosis liver; pericardial effusion; splenomegaly; auricular fibrillation.	Jaundice; purulent bronchitis; coarse cirrhosis; hyalisation of wall of rt. ventrical; ova in appendix; small abscess rt. kidney.	Spleen 500 gm. s. nodules; veins healthy.	Hyaline	Thickening	+	++	Atrophy	+	Slight Monocytic Lymphocytic	?	P = +	P = +	+	Fibrillary increase +++ Collagenisation ++
E.8	Specimen of Dr. McMichael from a case of Egyptian splenomegaly.						Hyaline	Thickening	+	++	Atrophy	++ Littoral reaction	Monocytic Lymphocytic	++	Nil	Nil	Slight	Fibrillary increase = ++

Peri-trabecular infiltration = Peri-trabecular infiltration with inflammatory cells.

COMMENT.

The splenic enlargement varied in this series of cases from 310 to 2000 gm. Extreme enlargements of 2000-5000 gm. are however recorded by Onsy (1937) in chronic cases. The enlargement varies with the chronicity and intensity of infection. There is reason to believe that the early acute enlargement met with soon after the cercarial invasion gradually dis-:appears if the infection is mild, but with repeated reinfection the enlargement becomes persistant and chronic. In the earlier stage (E.4) the capsule was thin and stretched and the consistency of the organ was soft and on section the surface was bulging and had a velvety appearance and a pinkish red colour. Subcapsular haemorrhages were met with. In chronic cases (E.6, E.3) the capsule was thickened and opaque and small patchy areas of perisplenitis were met with; on section the organ was firm and presented a flat brownish red surface in which the trabecular markings appeared as distinct greyish white lines. Siderotic nodules were not uncommon in this series. The appear-:ance of the malpighian bodies varied: in one case they were prominent and distinct, but in others they were atrophied and indistinguishable. In chronic cases the splenic artery at the hilum was markedly hypertrophied while the splenic vein was also thickened.

In/

In many cases spleniculi were met with suggesting a tendency of the splenic tissue to proliferation. In none of these cases was splenic or portal thrombosis or thrombophlebitis met with.

The appearance of the liver in 4 of these cases was more suggestive of a coarse nodular cirrhosis similar to the "toxic" type of Mallory (1911) rather than the definite clay pipe stem cirrhosis of bilharzial origin described by Symmers (1900). Girges (1930) has described the lesion as a diffuse hepatitis followed by a multilobular cirrhosis in the hepatic types of Sch. mansoni infection and argued that the cirrhosis is a diffuse toxic lesion and not one due to the local deposit of ova which caused the rarer type of pipe-stem cirrhosis. Similar results are recorded by Ferguson and Fairley (1920). In experimental bilharziasis in monkeys Fairley (1920) found that the early lesion was a diffuse hepatitis suggesting a circulating toxin. In the later stages bilharziomata were met with in association with a nodular cirrhosis. A characteristic feature was the presence of bilharzial pigment in the Küppfer cells in the later stages of infection.

In the present series of cases, the mesenteric nodes were generally enlarged and in one case had suppurated. A marked increase in the mononuclear non-granular cells of the bone marrow have been recorded/

recorded by Madden (1923) and Onsy (1937).

With regard to the association of splenomegaly with other bilharzial lesions numerous observations exist to show that either or both types of the parasite but often ~~xx~~ Sch. mansoni and the parasitic lesions such as early eosinophilic clusters around ova, bilharzial tubercles, adenopapillomata and fibrotic patches may be demonstrated by careful examination, in the organs of predilection.

With regard to the histology there is little definite knowledge regarding the changes in the spleen during the acute stage following carcarial invasion in man. However, Fairley (1920) has described the changes in monkeys as consisting of congestion and atrophy of the pulp, with increase in size of the malpighian follicles, irregular haemorrhages into the pulp and the presence of a finely granular bilharzial pigment both intracellular and lying free. These early stages showed more or less diffuse reactions with diffuse macrophage activity while localised lesions due to the deposit of ova were not met with. Onsy (1937) claims that the early lesions in man are not toxaemic but due to the primary invasion of the splenic parenchyma by the ova of the parasite. On the other hand Fairley, Ferguson and their associates (1923) do not find any evidence for a parasitic in-

:vasion/

:vasion of the branches of the splenic vein in both species and hold that focal lesions due to the deposit of ova are not common in the spleen and pancreas. The same difference of opinion exists with regard to the histological changes met with in the subacute and chronic stages. Thus in experimental studies in mice Brumpt and Chevallier (1931) have found massive deposits of ova in the spleen. The histopathological changes met with in the present series were diffuse rather than localised and bilharziomata were not met with in the spleen in any case.

The capsulo-trabecular system showed a variable thickening which became more marked with the chronicity of the lesion. With marked splenomegaly, the capsule became fibrous, thickened and hyaline and the elastic tissue and muscle fibres gradually disappeared. Tra-:becular thickening and splitting were commonly met with, but a much more significant reaction was the frequent presence of foci of trabecular inflammation, a "trabeculitis" similar to what is met with in spleno-:megalic cirrhosis. The fibrous tissue of the trabe-:culae had become spread out and invaded by numerous mononuclear cells and lymphocytes; occasionally fibro-:blasts and new capillaries grew in from the surrounding blood vessels to form a granulation tissue. Siderotic nodules due to old haemorrhage were sometimes present. Recent/

Recent haemorrhages without any definite deposit of blood pigment were also met with in the trabeculae. The trabecular veins and their beginnings in the pulp veins were free from inflammatory changes affecting the lining endothelium. The lining cells were flat and their nuclei thin and drawn out. Parasites were not met with within the vein nor was there any deposit of ova in the walls of the vein similar to that des-:cribed by Symmers (1904) in the hepatic branches of the portal vein in "pipe-stem" cirrhosis. Probably they could be discovered if present only by serial sections or by the method of digestion, or maceration of the soft tissues.

The malpighian follicles showed generally an atrophy of the lymphoid tissue though in one case hyperplastic changes were also met with. The cells of the follicles were generally small and medium sized lymphocytes, though occasionally large germ centre cells were found in the centre of the follicle forming large pale staining nodules containing irregular clusters of yellowish brown bilharzial pigment. The eccentric arterioles and their penicillar branches sometimes showed well marked peri-arterial fibrosis as shown in Fig.I. The areas of fibrosis sometimes extended round the follicle as demilunes of fibrous tissue. More often the pre-follicular arterioles were surrounded by dense bands of fibrous tissue in which/

which a fibrocytic spread from the adventitia of the vessel could be demonstrated. Haemorrhages were not common, and congestion of the marginal zone was infrequent.

The sinuses generally showed marked proliferative activity. The change known as "sinus hyperplasia" whereby an increased number of sinuses appeared in an area of splenic tissue was sometimes met with. This has been regarded as due to the mechanical stress of chronic venous stasis (Klemperer 1936) but congestion was variable and not marked. The littoral cells showed hyperplastic changes and were often projecting into the lumen. The appearance was not unlike that of a proliferating gland tubule rather than a distended blood vessel. Many free mononuclear cells, eosino-:philes and lymphoid cells were present in the lumen.

The pulp cords were thinner than normal and the loose syncytial mesh of the pulp had become altered to a more compact tissue in which the syncytial nuclei appeared oval and elongated and often fibroblastic in type.

Side by side with these changes affecting the sinuses and the pulp cords the free cells appeared grouped together in irregular foci in the pulp mesh. These cellular accumulations were most marked at the termination of the arterial capillaries in the pulp. Clusters/

Clusters of mononuclear cells, plasma cells, lymphoid cells and eosinophiles were grouped together in peri-:vascular foci which were also peculiar in the presence of brownish yellow clumps of bilharzial pigment. In two cases, the eosinophilic reaction was very marked and the sinuses as well as the pulp mesh appeared packed with these cells, a picture that is not unlike what has been met with in allergic reactions due to the presence of disintegrating parasites in filamiasis. Erythrophagocytes were not infrequently met with. Megakaryocytes were rare. Myeloid metaplasia was not met with in any of the series.

The fibrillary reticulum showed a well marked increase both in the interlacing fibrils of the pulp as well as in the encircling tangential fibrils of the sinuses (see Fig.5). Increased branching and thicken-:ing of the fibrils could be demonstrated by the Foot-Wilder stain. With Van Gieson's stain here and there some of the fibrils in the pulp had become markedly swollen giving the red fuchsin stain of collagen (see Fig.6). This change was quite distinct from the fibrous spread from the capsule and trabeculae and was a direct alteration of reticulin into collagen. Some of the malpighian follicles showed a peri-arterial fibrillary increase, but the change was not uniform. A spread of fibrils from the capsule and trabeculae could/

could also be demonstrated.

The bilharzial pigment that was deposited was found both in intracellular clusters and extracellular clumps and granules lying free. When intracellular they were inside large mononuclear cells some of which appeared loaded with pigment. Phagocytosis was not however so active as in malarial infections. The appearance of the yellow ochrous granules was not unlike malarial pigment, but the distribution was different. Unlike malarial pigment which is found uniformly throughout the whole spleen pulp in subacute infections from parasitic localisation in the mesh, bilharzial pigment seemed to be collected more around the terminations of arterioles though smaller granules had a more irregular distribution through diffusion. The appearance of the pigment itself was that of irregular blocks, clumps and finer granules somewhat like carbon particles. It was not crystalline nor in the fine maorphous granules that characterise haemo-:siderin. Chemically it did not give the Prussian blue reaction for free iron. It was soluble in ammonium sulphide, lithium carbonate and alkalies and alcoholic potash, just as malarial pigment. With regard to the origin it appears to be similar to the collections of altered blood pigment that are found in the intestinal caecae of the adult worms and pre-:sumably/

:sumably excreted or liberated by the destruction of the worm since Fairley (1920) has found it in the spleen in the early toxaemic stage of infection in monkeys.

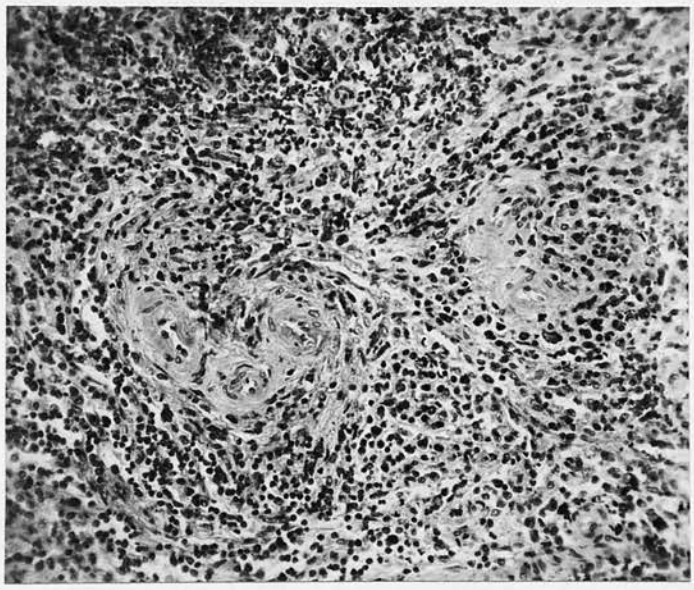

Fig.1. Egyptian spleen showing typical periarterial fibrosis. (x 200). H and E.

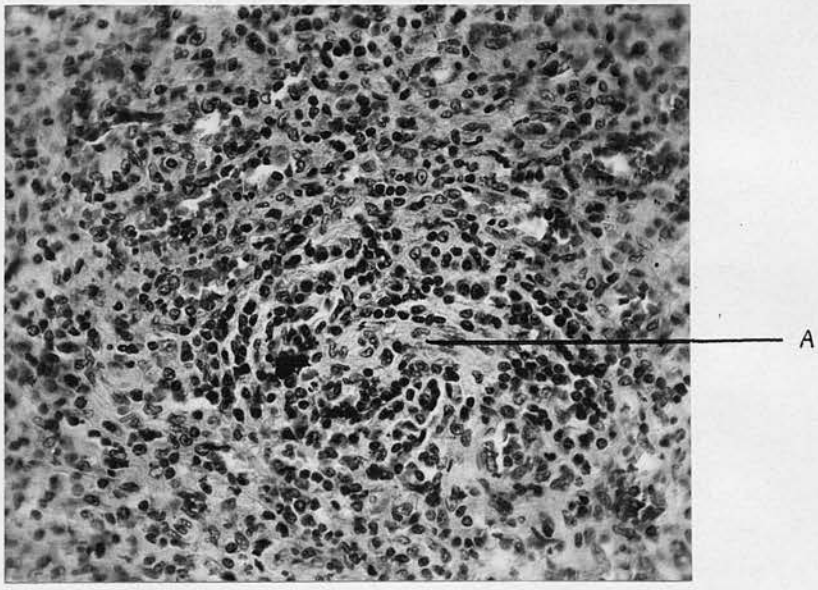

Fig.2. Egyptian spleen showing focal collections of pigment and focal cell infiltration round a small arterial capillary (A). (x 200).

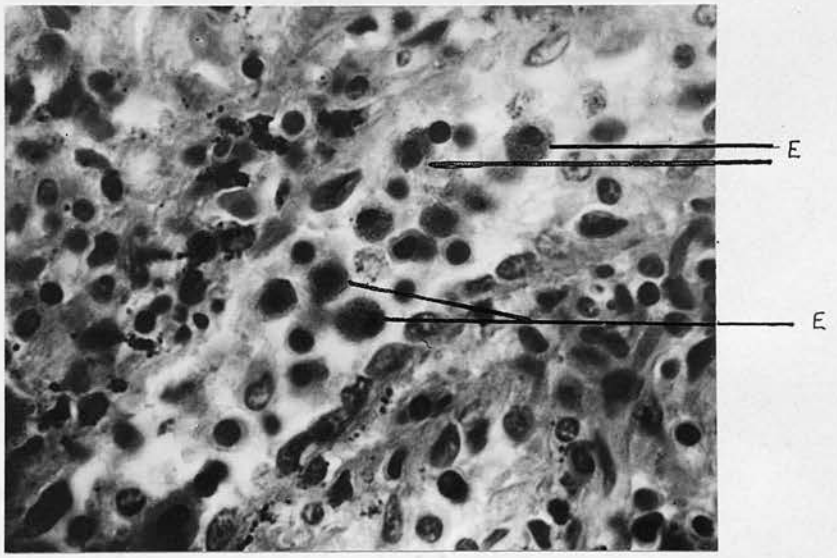

Fig.3. Egyptian spleen showing numerous eosinophiles (E) in a sinus. (x 750). H and E.

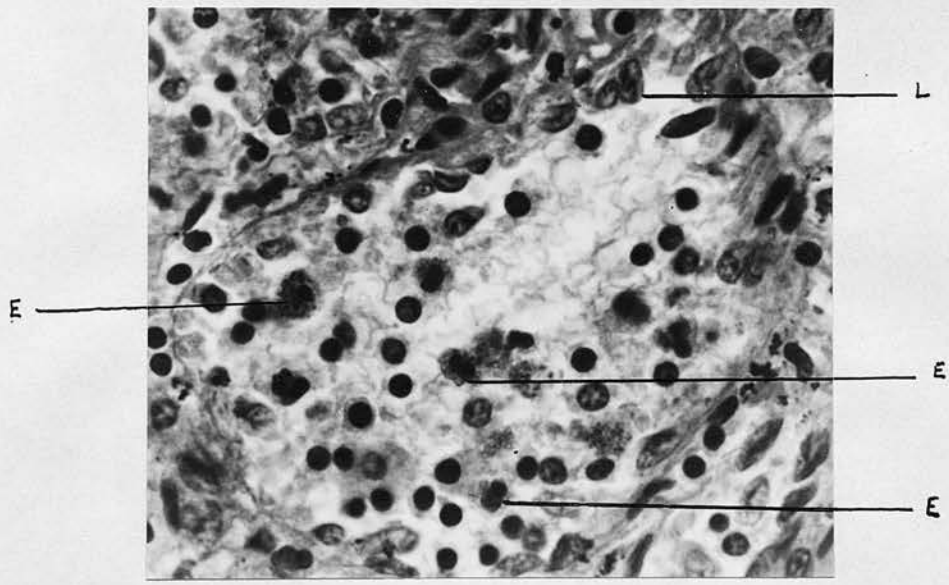

Fig.4. Egyptian spleen showing the littoral reaction; the lining cells (L) are projecting into the lumen; numerous free eosinophiles (E) are inside the lumen. (x 750). H and E.

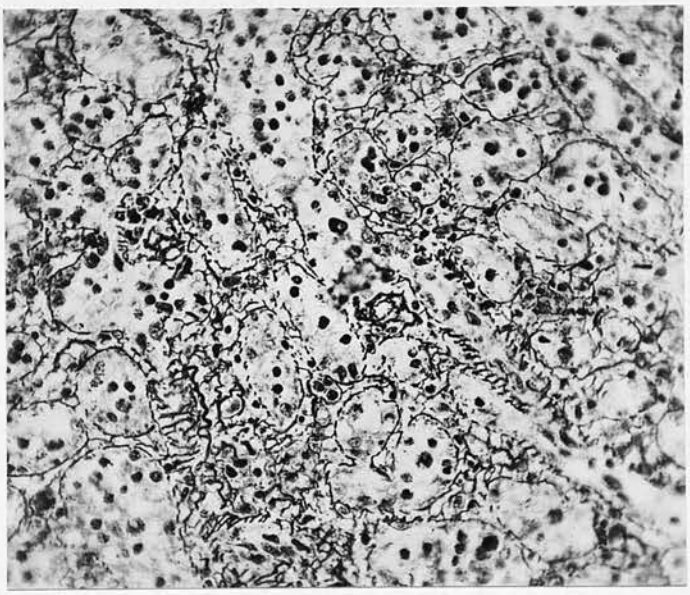

Fig.5. Egyptian spleen showing early "fibro-adenie" of the pulp; the fibrils are thin and there is little collagenisation. (x 300). Foot-Wilder.

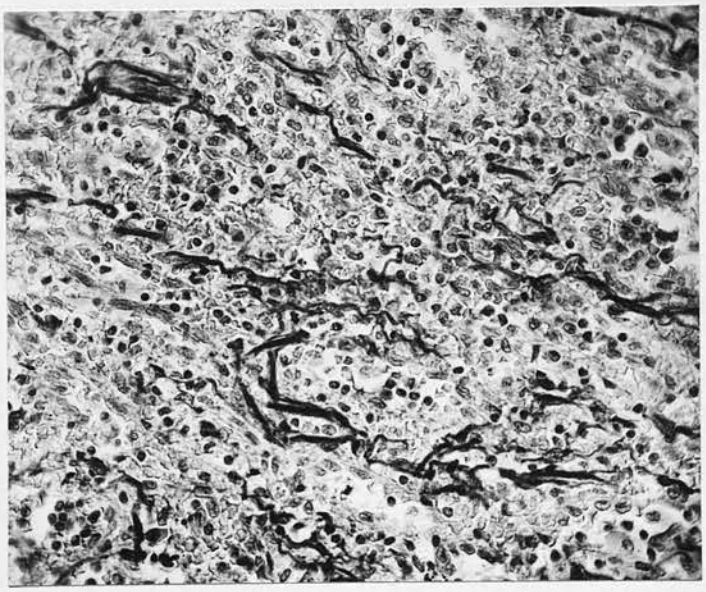

Fig.6. Egyptian spleen showing transformation of the reticulum threads into coarse collagen fibres (black). Anderson's iron haematoxylin and Van Gieson. (x 300).

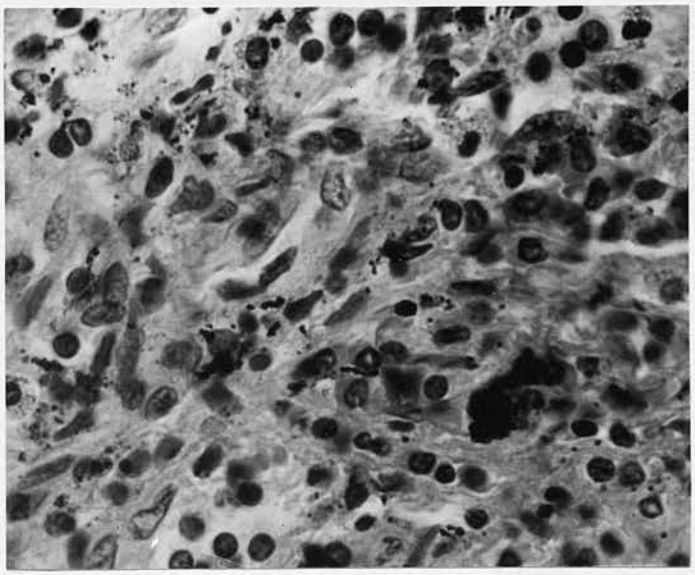

Fig.7. Egyptian spleen showing the morphology of bilharzial pigment. It forms irregular clumps, often extracellular, and finer granules lying free in the pulp mesh. (x 750). H and E.

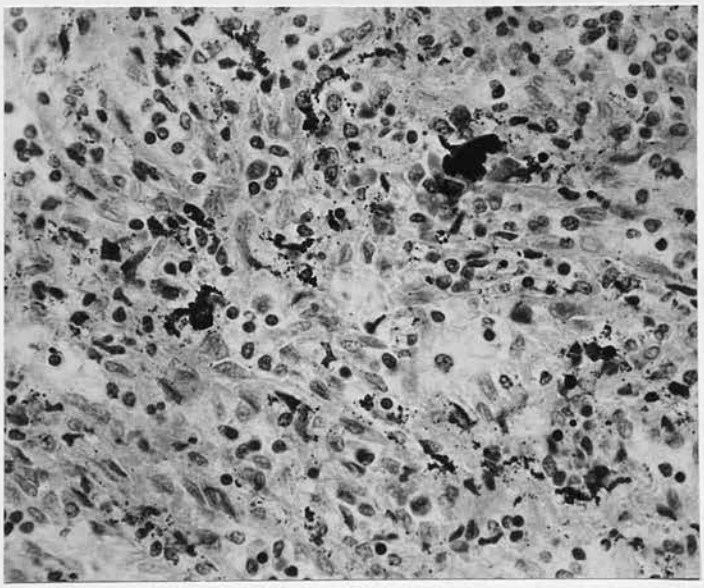

Fig.8. Egyptian spleen showing the "fibroblastic" alteration of the syncytial nuclei; there are also irregular clusters of pigment. (x 400). Leishman.

DISCUSSION.

In reviewing the different theories with regard to the pathogenesis of the splenic enlargement it is worthy of note that clinical and microscopic evidence obtained at operation and at autopsy on cases of splenomegaly does not lend any support to the view of Day (1933) of increased portal pressure as a primary causative factor. The splenic enlargement is quite marked in the earlier stages of the disease when at operation small microscopic focal lesions are met with in the liver, lesions which could not be regarded as causing any obstructive effect on the portal circula-:tion.

With regard to the possibility of a verminous phlebitis or pylethrombosis there is little evidence of any lesions in the portal veins, even though the pioneer studies of Leiper (1915) have demonstrated that the parasites live and mature in the portal vessels. Bernard Shaw (1937) in autopsy reports has carefully studied the portal vein in the four cases included in this group and has not recorded any thrombophlebitic lesion, though splenomegaly is extreme in one case. Aidaros (1937) with considerable autopsy experience in Egypt remarks that the spleno-portal venous system is invariably opened up in autopsies in Egypt/

Egypt in a search for the parent worms, and that thrombotic lesions are very seldom met with. Onsy (1937) mentions that thrombosis of veins and arteries do not occur in Egyptian splenomegaly. It is of interest in regard to this question that Fairley (1920) in experimental bilharziasis in monkeys has found the development of spleno-hepatic syndromes, but never any proliferative changes in the portal vein. Further in the present series of cases a histological examination of the branches of the splenic vein in the trabeculae has failed to reveal any significant lesions in the endothelium or subendothelial tissue. Escaped ova would naturally be deposited in the splenic vein if there is a rise of portal pressure but such a deposit is seldom met with in the spleen, but more often in the liver where the portal vessels ramify. Occasion-:ally the splenic artery appeared hypertrophied and the walls of the splenic vein thickened indicating an increased functional activity of the spleen or an increased vascular supply to a chronic inflammatory stimulus.

If one were to consider the possibility of splenic localisation of the parasites one has to take into account the findings of most workers (Ferguson and Fairley, 1920; Day, 1937) that the parasites are seldom met with in the splenic vein or its branches except in cases/

cases with advanced portal obstruction from cirrhosis where a collateral venous circulation had formed and the parasites swept off even into the lung (Day, 1937). On the other hand Perry's and Onsy's findings of ova in the spleen (Perry, 1925; Onsy, 1937) suggest that the parasites occasionally wander into the spleen against the vascular stream as in other organs. In Sch. japonicum infections Faust and Meleny (1925) hold that a splenic localisation with deposit of ova in the spleen is common. An entrance via the arterial channels is precluded by the presence of an extensive capillary system in the lung which would arrest the ova. In any case the infrequency of the finding of ova in the spleen suggests that heavy infections with ova are not common and that there is some other factor that is partly responsible for the diffuse splenomegaly.

Evidence for an allergic inflammation in the spleen in some cases of Egyptian splenomegaly is shown by the marked concentration of eosinophiles in the splenic sinuses and in the pulp mesh. Though the presence of the parasites in the vascular system induces a well marked eosinophilia in the blood in the earlier stages of infection as shown by the studies of Day and Ferguson (1909), Day (1911), Girges (1932), Onsy (1937) and other workers, the concentration of eosinophiles in the spleen in some cases is so marked as to suggest

a/

a local reaction. The studies of Campbell, Drennan and Rettie (1935) have shown the association between eosinophilia and allergic reactions and it would appear that in some of these cases there is a splenic allergy to some foreign protein in the blood. Preliminary experiments with egg white and horse serum injected in very small doses into the spleen in guineapigs have shown (Menon, 1937) that the response in the sensitised animal is characterised by a dilatation of the sinuses, leucostasis, local eosinophilia, and marked proliferation of the syncytial nuclei of the pulp, a response that is much more intense than that in the non-sensitised animal. In Egyptian splenomegaly there is not only the eosinophilic reaction (see Figs.3 and 4) but the littoral cells lining the sinuses show marked hyperplasia and differentiation. Onsy (1937) argues that the cellular reactions in the spleen are due to deposition of ova which are absorbed with extraordinary activity. He has been able to demonstrate ova in the spleen but the histological picture shown by his photomicrographs is that of bilharzial granulomata around the ova. Onsy holds that the rarity of finding of ova in the spleen is due to the rapidity of absorption by the macrophages. On the other hand, there is evidence in Symmer's description of bilharzial cirrhosis (Symmers, 1904) that ova that are deposited in the liver/

liver are not rapidly absorbed by the macrophages, but that the shell remains for a considerable time even though the embryo within the shell has been completely digested. The appearance of bilharzial granulomata in other organs as described by Fairley (1920) is that of local eosinophilic clusters in the earlier stages, followed by the formation of pseudo-tubercles with giant cells around the ova and slow encapsulation and fibrosis when the contained embryo is dead. There is also no evidence that the spleen has special powers to destroy with great rapidity large animal parasites except by the ordinary processes of foreign body reactions and encapsulation. It is noteworthy that in hydatid cysts in the spleen the material remains unabsorbed for a considerable time even as in the liver and other organs. Further the histological feature of the Egyptian spleen is that of a diffuse process rather than localised granulomatous formations as in tubercu-:losis and Hodgkin's disease. In experimental bil-:harziosis in monkeys Fairley (1920) has pointed out that a diffuse proliferation of the macrophage tissue is what is met with in the spleen rather than local lesions. Besides, the early splenic enlargement is found at a stage where in experimental infections deposition of ova had not commenced. The gradual increase and persistence of this enlargement with repeated/

repeated infection suggests that there is a definite association between the presence of the parasite, their toxins or products of disintegration in the vascular system and the splenomegalic process.

The behaviour of the spleen in acting as a bac-:terial and protozoal filter for the blood is shown by its reaction in acute bacterial infections such as enteric fever, and in protozoal diseases like malaria and kala-azar. The studies of Rich (1935) have shown that foreign proteins are also filtered from the blood and excite well marked cytological reactions. In bilharziosis, the presence of the parasite in the vas-:cular system presupposes the presence of excretory products, or abnormal protein derived from the secre-:tions or from disintegration of the ova or the parasites themselves. Evidence that these abnormal products are filtered by the spleen pulp is found in the perivascular collections of bilharzial pigment as well as the perivascular cell accumulations that are found in almost all cases. With regard to the question of actual <u>toxins</u> secreted by helminths McCoy (1935) holds that there is no definite evidence for the pre-:sence of exotoxins as in tetanus and diphtheria and that endotoxin set free by death or disintegration of the parasites may have possible effects on the host. Taliaferro (1929) also considers that the effects on the/

the host are due to the products of disintegration of the parasites. In any case the presence of abnormal protein around parasites or parasitic lesions causing allergic reaction is well known in hydatid disease, in filariasis and a whole series of helminthic infesta-:tions. It is thus possible to consider the spleno-:megalic process as a foreign protein reaction due in part to the products of disintegration of the worm or their ova in the circulation, and in part to a deposit of ova in the spleen. It is also evident that, with repeated flooding of parasitic protein, a state of allergy may be induced and result in a "hyperergic" response. The predominance of the lesion in the spleen is possibly due to the effect of the reticulo-endothelial mesh in acting as a filter for abnormal products in the blood. In the later stages of portal cirrhosis due to the associated damage to the liver, signs of portal obstruction would supervene and a congestive factor would come into play.

With regard to the problem of the association between the splenomegaly and the hepatic type of schistosomiasis in contrast to the commoner intestinal or pelvic type it seems probable that destruction of the parasites would be more common in cases with hepatic localisation, possibly from a higher antibody content in the portal vein. It is also well known that the parasites/

parasites are found only in scanty numbers in such cases and Girges (1932) believes that only the males are found. The theoretical implication would be that cases of splenomegaly are those showing a higher degree of resistance to infection in that many para-;sites especially the more slender females or their ova are destroyed in the vascular system and that during the course of their destruction liberate large amounts of foreign protein so that allergic reactions are induced in the spleen.

SUMMARY.

1. A study of Egyptian splenomegaly has shown that the splenic enlargement occurs at an early stage, is progressive and independent in the early stages of any portal hypertension caused by fibrotic lesions in the liver.

2. An analysis of the histopathological changes in 8 cases of Egyptian splenomegaly has shown that thrombosis or thrombophlebitis of the portal vein does not play any part in this splenomegalic process.

3. The histopathological changes suggest a diffuse inflammatory process characterised by macrophage activity and reactions around deposits of bilharzial pigment which have a perivascular distribution.

4. Occasionally eosinophilic reactions are met with indicating a local allergy.

5. In the later stages, marked fibrillary increase and fibrosis give a picture that is very similar to Banti's "fibro-adenie".

6. The theory is put forward that the splenomegalic process is due in part to a local allergic response to products of excretion or disintegration of the worms or their ova that are filtered by the spleen pulp. A local deposit of ova would also contribute to the splenic enlargement by a marked local tissue response.

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APPENDIX.

A Leishman Stain for the Spleen.

Staining tissues by the Romanowsky stain has very many advantages over the eosin methylene blue method of Mallory (1904) since cytoplasmic granules and intra-:cellular protozoa are brought out in very clear detail, provided the stain can be kept on the section through the process of dehydration. Modifications have been used especially with the Giemsa stain (Wolbach, 1922) to bring out intracellular parasites and inclusion bodies in sections, but uniformly good results are difficult to obtain. Turnbull (1931) has devised a method of staining with the Jenner stain adjusted to a pH value in such a way that the contrast between the red and the blue is brought out. In working with sections of the spleen with all these modifications, it was found that staining with the Leishman stain gave a very good contrast and all the ranges of colour in a blood film, provided the stain was not washed out during the process of dehydration with alcohol or acetone.

It was found that if the tissue was fixed in Helley's fluid, better results were obtained than by fixation with formol saline though Jöres fluid gave good results. The great difficulty with the Leishman stain/

stain when used for tissues was to get critical differen-:tiation, since staining as for a blood film had the effect of overstaining the basophilic elements while acidophilic and neutrophilic material was not brought out in contrast. It was found that immersion of the stained section in a 1 in 4000 solution of acetic acid in distilled water had the effect of bringing out the acidophilic elements in contrast to the basophilic nuclear material. The technique used was as follows:

I. Treat sections fixed in Helly's fluid with alcoholic iodine and afterwards with 2 per cent hyposulphite to remove the sublimate crystals.

II. Flood the slide with distilled water to remove the "hypo".

III. Stain by flooding the slide with a freshly prepared mixture of Leishman's stain and distilled water in the proportion of 1 : 1.5

IV. Overstain the section for 15 to 20 minutes without allowing the stain to dry.

V. Wash off the stain with distilled water.

VI. For differentiation, immerse in a Petri dish containing 1 in 4000 acetic acid till the section becomes definitely pink. This takes about 1 to 2 minutes.

VII. Transfer to another Petri dish containing distilled water to wash off the acid.

VIII/

VIII. Wipe the edges dry and blot the section almost dry between filter paper. This is the most important part of the technique as dehydration with alcohol is made as short as possible.

IX. Pour two drops of absolute alcohol on the section. It will be noticed that some of the stain comes away during this process. The whole dehydration should not take more than a second.

X. Immerse the slide in Benzol or neutral xylol.

XI. Mount in Gurr's neutral mounting medium.

Note:- The stain is not quite suitable for purposes of photo-micrography as the different shades of blue and pink are not well defined in black and white.

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